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A STUDY OF THE FUNGOUS FLORA OF WHEAT ROOTS[1]

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Previous work conducted by the senior author and colleagues (15) and others has shown that the root system of wheat is rather extensive at the lower levels. The isolation of fungi from wheat roots growing in the surface soil layers and from the base of culms has been made by many investigators. The significance of some of the fungi obtained from such sources in causing rootrot of cereals is well known. The problem before us in the present work was to attempt a survey of the prevalence of fungi in or on wheat roots at the various soil levels; and, in so far as possible, to interpret their significance in reference to root diseases.

REVIEW OF LITERATURE

The literature on soil mycology in its many phases is extensive. In the present paper our literature review will be confined chiefly to investigations pertaining to the association of certain micro-organisms with plant roots. As roots of most plants are not by any means confined to the upper soil layers, the vertical distribution of fungi must be considered. Taylor (19) in making isolations from the soil obtained *Fusaria* from a depth of 24 inches. Rathbun (12) isolated various fungi from the soil to a depth of 44 inches. Both investigators attribute the distribution of soil fungi, in part at least, to the activity of grubs and earthworms. Starkey's (16, 17, 18) extensive experimental work and literature review brings forth abundant evidence to show that, as a general rule, soil micro-organisms are most plentiful in the vicinity of plant roots. The abundance of fungi in material from the superficial layers of roots, as compared with soil material a short distance away, was striking. Fewer fungi were obtained by Le Clerg (10) from the lower soil levels than from the surface. Some species, however, were isolated from the 6-foot level. Further evidence that bacterial and mould activity is greater on or near plant roots was shown by the work of Thom and Humfeld (20); they observed that in either strongly acid or strongly alkaline soils, the roots, in this case corn, maintained a zone in their immediate vicinity with a reaction approximating neutrality. This area was consequently very favourable for microbial activity. Root material from tobacco plants attacked by rootrot

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gave higher counts of micro-organisms than similar root material from uninfected plants. Fellows and Ficke (4) found *Ophiobolus graminis*, a wheat rootrot fungus, to occur at a depth of at least 15 inches. Greatest injury resulted when roots were infected in the upper levels near the seed. The investigations conducted by Bisby and co-workers (2, 3) reveal the common occurrence of fungi, including some parasitic forms, in the soils of Manitoba. Timonin (21) also working with Manitoba soils, reports that the number of fungi decreased at the lower depths of the soil profile. Anaerobic bacteria and fungi were more prevalent at the lower levels.

Pathologists working on root diseases have lately been giving some attention to fungi found within the roots, as well as to those forms obtained by isolation from root material or from the soil. The common occurrence of a mycorrhizal type of fungus in the roots of crop plants, particularly legumes, has been reported by Jones (8). The importance of direct examinations, to determine the number and kind of fungi in the roots, along with isolation studies have been stressed by pathologists working on root diseases of strawberry, raspberry and tobacco, namely Truscott (22), Hildebrand (6), Koch (9), Hildebrand and Koch (7) and Berkeley (1). The mycorrhizal type of endophyte was found to be a prevalent inhabitant of the roots of these plants. A new fungal parasite found in the roots of wheat grown in eastern Canada has recently been reported by Ledingham (11). Its pathogenic significance has not been fully determined.

MATERIALS AND METHODS

Wheat plants from which material was to be taken for isolation and examination were carefully excavated by the Weaver (23) method. The work was conducted in 1935 and 1936. Specimens were taken at Saskatoon and Indian Head, Saskatchewan, and included samples obtained during the seeding stage (June) and at a period just prior to maturity (August). The plants selected were growing under ordinary crop conditions. The roots were carefully traced so that definite material could be selected for each level. The following specimens were collected in each case:—*(a)* sub-crown internodes, *(b)* crown root pieces, and *(c)* seminal root pieces from near the seed, all of which were within the first six inches of soil; *(d)* pieces were then taken at the 1-foot level with additional samples at each foot to the depth of root penetration. As the roots were excavated, the proper pieces were selected as soon as they were exposed and placed in a clean envelope. No effort was made to take only portions bearing lesions and for the most part conspicuous lesions were absent below the first foot. Comparable specimens were taken at the same time and preserved for future study. If the samples to be used for isolation work had to be mailed, they were first allowed to dry, away from direct sunlight; otherwise they were plated the same day as collected. The specimens were rinsed in tap water, cut into suitable lengths and then washed thoroughly in sterile water (14). They were immediately transferred to potato dextrose agar plates after being washed and were incubated at room temperature.

The soil of the two locations mentioned were quite different. According to information obtained from Dr. J. Mitchell of the Soils Department, University of Saskatchewan, they may be briefly described as follows:

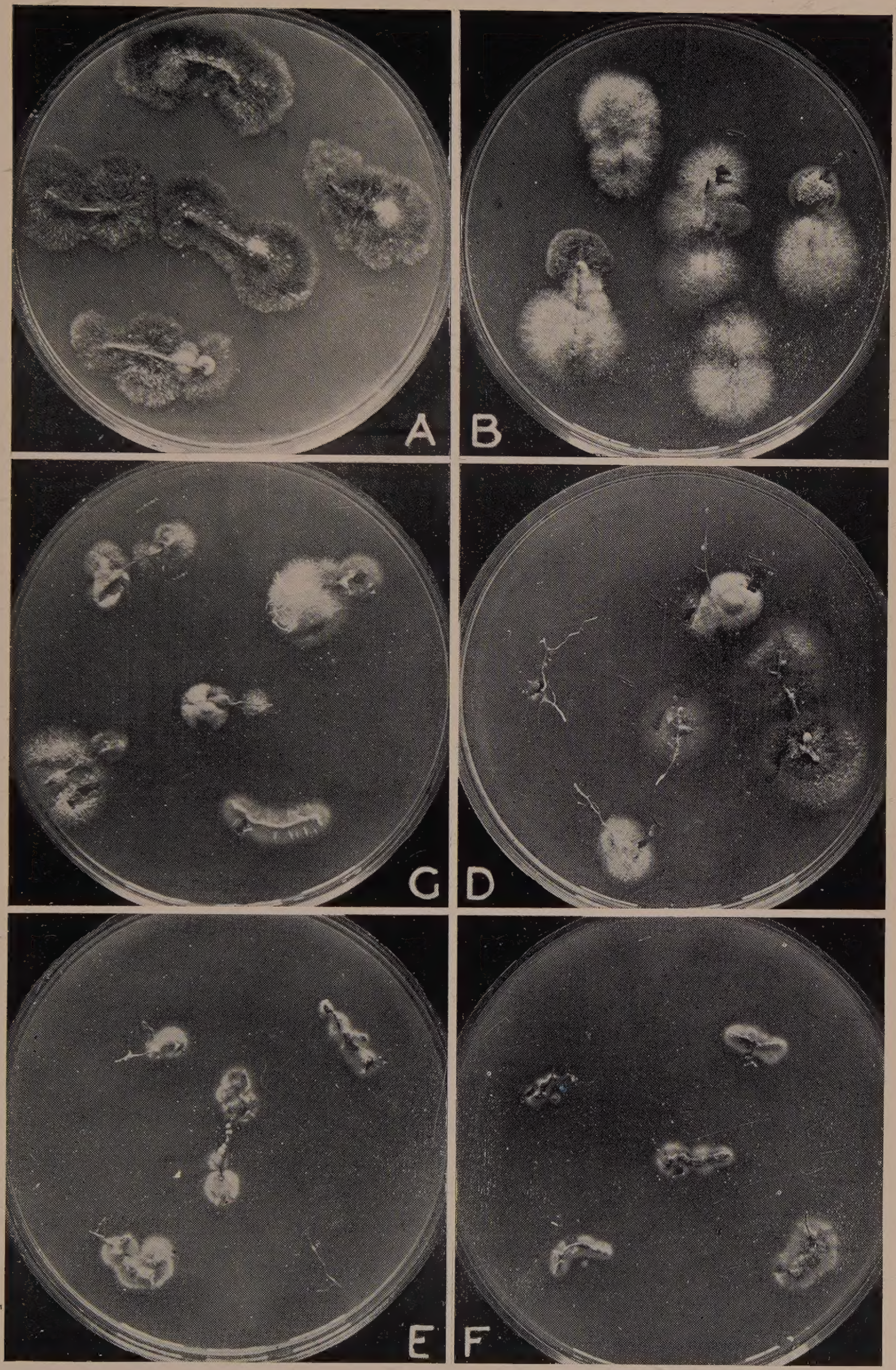

FIGURE 1. A representative group of plates showing the various fungi growing from the isolation pieces onto potato dextrose agar. (A) Subcrown internode, (B) Crown roots (C) Seminal roots near seed, (D) (E) and (F) Seminal roots at the first, second and third foot levels respectively. The specimens were collected from wheat plants excavated at Indian Head in August, 1936.

Saskatoon—"A dark brown, light loam soil of the Plains-Park transition zone. The profile has the dark brown soft cloddy surface, the brown long cloddy rather hard sub-surface, and the gray calcareous subsoil usual for the well-drained soils of this zone. The topography is undulating." *Indian Head*—"The surface soil consists of a very dark brown to black friable layer. The subsoil is more compact, somewhat columnar, underlain by a mixed brownish yellow and dark gray clay, cloddy in structure and high in lime. The topography is level to undulating."

ISOLATION STUDIES

The results covering the work for two seasons for each station have been combined. The number of isolates obtained from the roots of seedlings and mature plants were added to provide a total for each level. The combined data serve to emphasize the main objects of the work, which were to determine the species and relative abundance of fungi obtainable at the different levels. The total number of pieces for each level, from which isolations were attempted, approximate one hundred, except for the lowest depths where only a small amount of root tissue was available. The results are given in Table 1.

The tabulated results show the rather wide diversity of species. Considering total amounts, more fungi were isolated from the plant parts developing within the first foot of soil. There is a falling off, particularly after the second foot, but a fair number were isolated from the lowest depth of root penetration, namely four feet. Most of the species obtained from the lower levels were slow growing forms and it is possible that had they been present in material from higher levels, the fast growing species would have submerged them (Figure 1). On the other hand the rapidly growing fungi such as *Fusaria* were apparently not in or on the specimens from the lower depths. *Helminthosporium sativum* and *Fusaria* were the most abundant fungi isolated from the upper levels and only rarely were they obtained at or below the first foot. At the lower levels *Geomyces vulgaris*, *Botrytis terrestris*, *Botrytis* sp. and *Cylindrocarpon* sp. were rather common. Some other fungi such as *Colletotrichum* sp. and *Penicillium* sp. were present at most levels. Of the total number of isolates identified, roughly 40% were *Fusaria* and 10% *H. sativum*. Consequently well known wheat parasites or species with parasitic tendencies constituted around 50% of the isolates. Furthermore, *Colletotrichum* sp. which belongs to a genus containing forms parasitic on wheat accounts for another 7%. The next largest group include *Cylindrocarpon* sp. 6%. *Penicillium* sp. 6%, and *Geomyces vulgaris* 5%; the pathogenic significance of these, as far as cereals are concerned, is difficult to evaluate at present. The unidentified isolates account for about 13% of the total. The remainder contain some species which were found to be parasitic in laboratory tests. In addition isolations were made in 1935 from decayed root pieces, presumably old cereal roots, collected at the various levels. Generally speaking similar fungi were obtained from this material as from living roots. The following species isolated only from this source might be mentioned: *Fusarium sambucinum* Fkl. and *F. solani* (Mart) App. and Wr., from the first foot, and *Monotospora Daleae* Mason from the 2-foot layer.

TABLE 1.—FUNGI ISOLATED FROM THE SUB-CROWN INTERNODE AND ROOTS OF WHEAT PLANTS EXCAVATED AT SASKATOON AND INDIAN HEAD IN 1935 AND 1936

Number of fungi from different levels

Fungi	Saskatoon							Indian Head								Grand Total
	Sub-crown inter-node	Crown roots	Seminal roots				Total	Sub-crown inter-node	Crown roots	Seminal roots					Total	
			Near seed	1st foot	2nd foot	3rd foot				Near seed	1st foot	2nd foot	3rd foot	4th foot		
Mortierella elasson Sideris and Paxton			1	9			10		7	3					10	20
Mucor sp.			1	1			2				2	1			3	5
Zygorhynchus sp.		1					1									1
Acrostalagmus cinnabarinus Corda				2			2									2
Alternaria sp.								2			2			3	7	7
Botrytis terrestris Jensen				1			1				8	6	1		15	16
Botrytis sp.												5			5	5
Cephalosporium curtipes Sacc.													1		1	1
Colletotrichum sp.	1	6	8		2		17	1	2	4	6	17	6		36	53
Coniothyrium sp.									2	1					3	3
Cladosporium herbarum (Pers.) Link.			3	3	1	1	8	1				1			2	10
Curvularia sp.	1				7		8	1	3	4		2			10	18
Cylindrocarpon magnusianum (Sacc.) Wr.												4			4	4
Cylindrocarpon sp.					5		5			1	10	15	12		38	43
Dicoccum sp.				1			1									1
Epicoccum nigrum Link.			3				3			6					6	9
E. purpurascens Ehreub			1				1			1					1	2
Fusarium avenaceum (Fr.) Sacc.	2	4	5				11	1	21		1				23	34
F. concolor Reinking.											1				1	1
F. culmorum (W. G. Smith) Sacc.	2						2	2							2	4
F. equiseti (Corda) Sacc.	9	17	23	2			51	5	22	16	3				46	97
F. orthoceras App. and Wr.		1					1				4				4	5
F. oxysporum Schlecht.	1	5	8				14			2	1				3	17
F. oxysporum var. *aurantiacum* (Link.) Wr.		8	16				24	4	30	13	7	1			55	79
F. scirpi var. *acuminatum* (Ell. and Ev.) Wr.	7	13	9	2			31	4		1					5	36
F. sporotrichioides Sherb.										1					1	1
Fusarium sp.	1				9		10		3	9					12	22

Geomyces vulgaris Traaen.				2			2		1	2	8	14	6	3	34	36
Gliocladium roseum (Link) Bainier	4		1	1			6									6
Helminthosporium sativum P.K. and B.	20	3	3	1			27	40	5	2					47	74
Hormodendrum cladosporioides (Fres.) Sacc.				1			1									1
Metarrhizium sp.			1				1									1
Monilia implicata Gil. and Abb.			1				1									1
Penicillium lilacinum Thom.				5			5				1	1			2	7
P. restrictum Gil. and Abb.			1				1									1
Penicillium (coremoid)			2				2									2
Penicillium sp.	12	2	5	2			21	4	2	7	3	2			18	39
Pestalozzia sp.				2			2				1				1	3
Stemphylium sp.										4					4	4
Torula allii (Harz.) Sacc.		6			1		7				2		9		11	18
Trichoderma koningi Oud.		2	1				3		1						1	4
T. lignorum (Tode) Harz.	1		1				2									2
Verticillium nubilum Pethybr.					1		1				4				4	5
V. terrestre (Link) Lindau				1			1									1
Undetermined		1	2	30	14	12	59	7	3	3	16	5	5	7	46	105
Totals	61	69	96	66	40	13	345	72	102	80	80	74	40	13	461	806

PATHOGENICITY TESTS

All the fungi reported herein were tested in a preliminary way. They were grown on a soil medium fortified with 1% corn meal and moistened with a weak dextrose solution. When growth was well established the medium was transferred to petri dishes; then twenty seeds thoroughly washed in sterile water were sown to each dish. After incubation at 24° C. for eight days, positive cases of root and coleoptile infections were very evident. Re-isolation was easily accomplished when desired. Wheat, oats and barley were used; the results are presented in Table 2. Fungi which gave negative reactions in this test were not listed.

TABLE 2.—INFECTIONS OBTAINED IN A LABORATORY TEST ON WHEAT OATS AND BARLEY SEEDLINGS WHEN INOCULATED WITH FUNGI ISOLATED FROM WHEAT ROOTS OR THE SUBCROWN INTERNODE

Fungus	Degree of infection on		
	Wheat	Oats	Barley
Fusarium orthoceras	Slight	Slight	Moderate
Fusarium culmorum	Severe	Severe	Severe
Fusarium avenaceum	Slight	Slight	Moderate
Fusarium oxysporum	Nil	Slight	Nil
Fusarium oxysporum var. *aurantiacum*	Nil	Slight	Nil
Fusarium sp.	Nil	Slight	Nil
Gliocladium roseum	Slight	Nil	Nil
Trichoderma koningi	Severe	Severe	Severe
Stemphylium sp.	Slight	Nil	Nil
Helminthosporium sativum	Severe	Slight	Severe
Cephalosporium curtipes	Severe	Severe	Severe
Colletotrichum sp.	Severe	—	—
Coniothyrium sp.	Severe	Slight	Slight
Pestalozzia sp.	Nil	Moderate	Moderate

It is of course realized that this was distinctly a laboratory test with conditions very favourable for the fungus. The plants did not develop beyond the very early seedling stage. The results show that fungi considered as major pathogens in causing common rootrot of cereals, such as *Fusarium culmorum*, and *H. sativum* were notably virulent. *Colletotrichum* sp. was classed as severe on wheat. Other species not so well known in root disease studies but which were virulent in this test, were *Cephalosporium curtipes* and *Trichoderma koningi*. Most of the fungi found to be parasitic were from the upper levels. The exceptions were *C. curtipes* isolated from the 3-foot depth and *Colletotrichum* sp. which was obtained from practically all levels. It is of interest to observe that none of the species were parasitic which were isolated frequently or almost entirely only from below the first foot.

HISTOLOGICAL OBSERVATIONS

When root pieces were collected for isolation purposes, some specimens from the various levels were preserved in formo-acetic-alcohol for later examination. This material was taken regardless of whether the roots at the respective level showed lesions or not. The samples in each case were of necessity small in number. A preliminary examination was made by

crushing the root pieces on a slide and staining with the usual lacto-phenol cotton-blue preparation. Some of the better preparations were destained by washing through ethyl alcohol, then re-stained with erythrosin and malachite green and mounted in balsam.

Observations showed that lesions visible to the naked eye were more common in the 1936 material than in the 1935 samples. Such lesions were always more conspicuous on the root pieces collected later in the season. Small discoloured areas, micro-lesions, chiefly in the cortex and observed only with the aid of a microscope, occurred in about the same frequency each year. These were also more common in the older specimens. Mycelium was invariably seen in roots showing either macro- or micro-lesions, while on the other hand mycelium was quite commonly found in roots which lacked distinct lesions. Some of the micro-lesions appeared to be of bacterial origin. The examinations revealed the presence of fungi associated with the roots at all depths including the 3-foot level which was the maximum depth at which specimens were collected. The subcrown internode and the crown roots near the surface frequently showed distinct lesions, along with the usual type of hyphae (probably *Helminthosporium sativum* and *Fusarium* sp.) associated with common rootrot infections. Very few lesions were seen below the first foot. The occurrence of mycelium in any great amount on the surface of the roots was rarely observed. Fragments of varying sizes of one or two types of dark mycelium were noticed rather commonly on the surface of roots at all levels, in the specimens collected both seasons. *Pythium* oospores of the browning rootrot fungus were seen in the Indian Head specimens, in slight amounts each year.

The direct examinations of specimens from the seminal root system revealed a phycomycete as the dominant fungus. This was in contrast to the prevalence of common rootrot fungi and their characteristic lesions on the crown roots and adjacent tissues. Common rootrot lesions were not found below the first 6 inches in the material examined whereas the phycomycetous type was not observed in the small sample of crown roots examined. In the seminal roots, however, this particular form of endophyte was observed at all levels. It was well established in some specimens, invading practically every cell of the cortex of some laterals for a considerable distance along the rootlet. There may have been more than one type but this could not be determined definitely. The mycelium was non-septate, rather large and well developed and ramified the cortical tissues passing from cell to cell without any distinct constriction in penetrating the walls. There was good evidence of appressoria in some of the epidermal cells indicating direct penetration. Moreover, the hyphae were commonly seen in root-hairs. The mycelium tended to mass in the inner cortical cells adjacent to the endodermis. It was not definitely observed beyond this barrier. In the cells where the mycelium collected in mass, the hyphae appeared to be surrounded with a granular material thus filling the entire cell. In some such cells the granular matter revealed traces of apparent old or empty hyphae (Figure 2). In a few cases, however, this formation upon closer examination seemed to be made up of fine hyphae, simulating somewhat the arbuscules depicted by certain

investigators studying mycorrhizal fungi in plant roots[3]. Although such infections, when well established, were very conspicuous yet there was no distinct tissue discoloration. The fungus was found in all collections of the seminal roots. The invasion was about the same in extent, for both the Saskatoon and Indian Head plants. The former grew on soil of average quality while the latter developed in soil of good fertility.

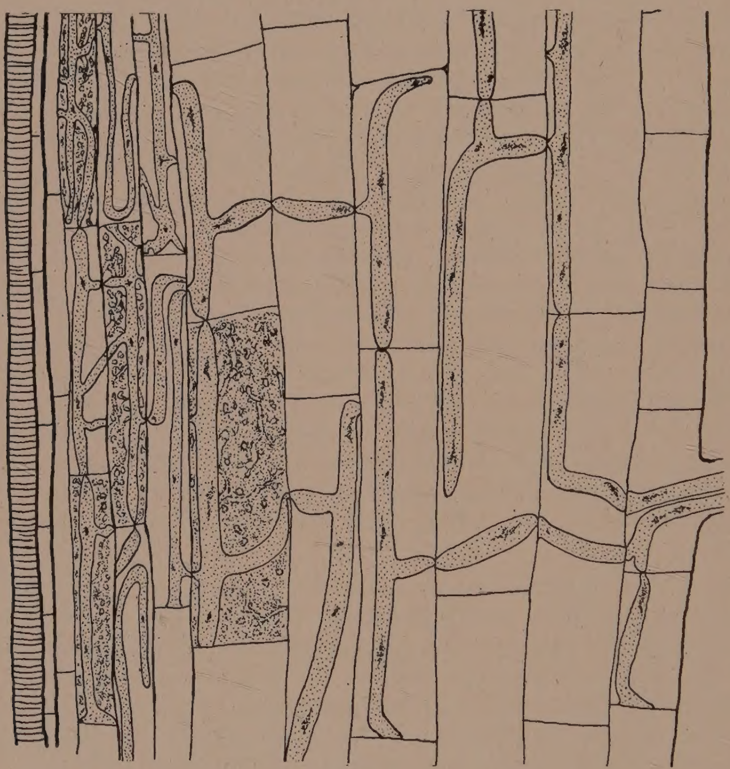

FIGURE 2. A composite drawing from specimens collected at Indian Head, showing an unidentified phycomycete well established in a portion of the cortex of a seminal root of wheat at the 2-foot level. The mycelium was inclined to mass towards the endodermis where cells filled with hyphae and granular material were observed. Proportioned with the camera-lucida. ×250.

DISCUSSION

It has been shown in the present investigation that a large and diverse group of fungi can be isolated from wheat roots. It is realized, of course, that many phycomycetes and most basidiomycetes are not obtained by the usual methods. The direct examination of preserved material, therefore, was depended upon to reveal other fungi, especially root invaders, which would not ordinarily be obtained by isolation methods. In an effort

[3] NOTE.—Dr. L. W. Koch, who was present at the meeting and who has had considerable experience in examining tobacco and strawberry roots, kindly consented to go over some of our slides. He expressed the opinion that the phycomycete found in the wheat roots was very similar in appearance to the one commonly found in tobacco in Ontario. The massed granular material in the inner cortical cells were arbuscules according to Dr. Koch. Many of these were in various stages of disintegration.

to eliminate as many of the distinctly soil forms as possible our isolation attempts were confined to root and subcrown internode material. The results from such isolations would then give a truer record of the fungi which invade or are associated in some way with the plant. It is this fraction of the soil flora which we assume may be of the greatest significance from a pathological viewpoint. The results of Henry (5) and Bisby (2) are of interest in this connection; they observed that *Helminthosporium sativum*, a well known wheat parasite, occurred rather infrequently in isolations from the soil. In the investigations under discussion, this pathogen was commonly isolated especially from the subcrown internode of wheat. Generally speaking, of the 44 identified fungi isolated, 15 or so can quite definitely be classed as pathogenes. On a basis of total isolates well over 50% belong to the pathogenic forms. These results indicate that a good proportion of parasitic isolates may be expected when isolations are made from plant tissues.

In regard to the vertical distribution, many isolates were obtained from the lower levels but very few were pathogenic. If this is the usual situation, it is of considerable interest, for a large part of the wheat root systems is found below the first 6 inches or first foot. In dry seasons especially, when the plant is largely dependent upon the seminal roots, invasions at the lower levels by such species as *Cephalosporium curtipes* or *Colletotrichum* might cause some injury. On the other hand, the common occurrence of pathogenic fungi in the surface layers as shown by isolations and lesions, is of definite significance. Within this upper zone, the main seminal roots meet at the first node and are connected with the crown by the subcrown internode. This portion of the axis must be considered of vital importance. A deep infection of the subcrown internode or proximal part of the seminal root system would certainly cut off the entire lower root system. Injuries of this nature have been observed in the case of infections by *Ophiobolus graminis* (15). Fellows and Ficke (4) found the same fungus to cause greatest injuries when inoculations were made at or near the seed. Furthermore the growth of crown roots and tiller buds takes place at the surface and within the upper soil layers. More isolates were obtained from the first foot than from below this level and most of these belonged to pathogenic species. Pathogenic fungi were, as a rule, rarely isolated from below the first foot. It would appear, therefore, that in problems of root diseases considerable attention must be given to infections of the plant parts found within the upper layers. It is not suggested, however, that the lower levels can be ignored, as information on infections at the lower depth is rather meagre. In dry season large and deep cracks appear in most prairie soils and these would allow easy access of surface organisms especially during heavy rains.

Direct examinations gave additional information on infections of the root system. The rare occurrence of lesions at the lower levels would appear to indicate lesser parasitic activity at these depths. On the other hand a phycomycetous infection was rather common below the first foot. This fungus was observed to be well established in portions of the cortex in roots of all orders. There were no ordinary pathological reactions at the infection foci, unless the granular cell contents mentioned above are considered as such. Discoloured cell walls or cell contents were not

observed. It is difficult to appraise the influence of such infections one way or the other. We are inclined to consider this endophyte as of the nature of a mycorrhiza somewhat similar to the type reported in papers reviewed above (8, 22, 6, 9, 7, 1).

SUMMARY

1. Isolations were made from the subcrown internode and roots of wheat plants excavated at Saskatoon and Indian Head in 1935 and 1936.

2. A total of 806 isolates representing 27 genera were studied. The results for the two years were totalled and tabulated to show the vertical distribution and frequency of occurrence of the isolates for each station.

3. More isolates were obtained from the first foot than from the lower levels, and over 50% of the total identified isolates were classed as pathogenic. Pathogenic fungi were rarely isolated from roots below the first foot. About 13% of the total number of isolates were undetermined.

4. Direct microscopic examination of preserved material revealed the rare occurrence of lesions on the roots below the first foot. A fungus considered as of a mycorrhizal type was commonly seen in many of the seminal root collections.

5. The pathologic significance of the results in studies of root diseases is briefly discussed.

ACKNOWLEDGMENTS

The authors wish to acknowledge with sincere thanks the help received from Dr. G. R. Bisby, formerly at the University of Manitoba, who identified most of the fungi, and also assistance from Dr. W. L. Gordon of the Dominion Rust Research Laboratory who determined the *Fusaria*. They wish also to acknowledge the assistance of Mr. B. J. Sallans who collected the Indian Head specimens.

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THE DEVELOPMENT OF A FLOTATION PROCESS FOR THE CLEANING OF CLOVER SEEDS

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FOREWORD

The problems of the seed cleaners were brought to our notice rather accidentally.

Mr. O. S. Hunter, of the A. S. Hunter Co., Durham, Ontario, came to us for material for an experiment he was making. In the course of conversation he told us much about difficulties in the separation of some kinds of seeds from others. Later, Mr. Hunter gave us considerable assistance by supplying information and material.

A few weeks later, Mr. A. W. Campbell, B.A.Sc., of the Eddy Seed Cleaners Ltd., came in to discuss the application to the mineral field of a centrifuge used in his business. In the natural exchange of ideas, we learned that the cleaning of seeds and their preparation for market was a considerable industry. We also heard more of the difficulties which existed in the removal from clover seed of some weed seeds for which there was no satisfactory process.

In the further pursuit of information on a problem that seemed to call for application of the principles of ore dressing, we called on the Hon. T. L. Kennedy, then Minister of Agriculture of the Province of Ontario. We discussed the problem with him in a general way and were referred to Mr. J. A. Carroll of the Markets Branch of the Department of Agriculture of Ontario.

Mr. Carroll told us the question of better grade seed was engaging the attention of the Seed Branch of the Dominion Department of Agriculture and consequently we met Mr. W. J. W. Lennox, District Inspector for Western Ontario of the Seed Branch of the Department of Agriculture of the Dominion of Canada. Mr. Lennox was intensely interested and offered to co-operate with us in any way possible, by giving information, supplying samples, analysing products, etc. We here acknowledge our indebtedness to Mr. Lennox and to Dr. C. W. Leggatt, Supervising Analyst of the Dominion Seeds Branch in Toronto. These gentlemen have given us every assistance, and in view of the considerable ignorance of two mining engineers about an agricultural problem, our work would have proceeded much more slowly without their co-operation.

Subsequently Mr. Lennox introduced us to Mr. Eddy of the Eddy Seed Cleaners Ltd., who opened up his plant for our inspection and study, and since then we have worked in close co-operation with Mr. Eddy.

The following report does not give a complete statement of the many experiments made but gives only summaries of them to indicate the nature of the experiments and the conclusions derived from them.

The investigation was carried on and is still progressing in the Laboratories of the Department of Mining Engineering, (provided through the instrumentality of the Hon. Chas. McCrea, then Minister of Mines) of the University of Toronto.

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[1] Associate Professor of Mining Engineering.
[2] Consulting Engineer. At one time instructor in Mining Engineering.

The finances were partly supplied by the School of Engineering Research of the University of Toronto and partly by private contributions.

THE CLEANING OF SEEDS

The Seeds Act of the Dominion of Canada which governs the sale of crop seed in Canada divides seeds into various classes and subdivides each class into three grades and a reject. Dealers are not permitted to sell rejects for seed purposes, and the market for Grade 3 is limited. For each grade the Act specifies the allowable numbers of contained weed seeds.

The particular class of seed with which this investigation deals is that of alfalfa and the clovers, *i.e.*, sweet clover, red clover (Figures 1, 2 and 3), crimson clover, alsike and white clover (Figures 4 and 5).

For this class of seed, Canada (more particularly Ontario, Quebec and Alberta) has a large market both for export and for home consumption. This market might be much greater were it not for weed seeds. According to Mr. Lennox "the need for improved seed cleaning methods is apparent from the enormous loss annually sustained by agriculture in the province of Ontario through the prevalence of weeds in the fields and of weed seeds in the grain and seed produced and used for seeding in the Province. Depreciation in market value of the seed crops because of weed seeds and other impurities which cannot now be removed probably amounts to between $200,000 and $300,000 each year. The annual production of alsike clover seed in Ontario averages more than 100,000 bushels and it is a conservative estimate that the crop is depreciated in value on an average of at least $1.00 per bushel because of weed seeds that cannot be removed effectively. Alfalfa and sweet clover seed production varies widely and averages 80,000 bushels per annum and much of the value of this is lessened because of such weed seeds as bladder campion and couch grass. Red clover seed production has decreased greatly within the Province, partly because it is impossible to clean a large proportion of the crop so that it will be marketable." . . . .

"The direct loss in depreciation of seed crops is only a part and a small part of the loss to agriculture sustained through weed seeds in seed crops. The standards under which seeds of the various grades may be sown under the Dominion Seeds Act are probably as high as it is now practicable to enforce; but there is wide distribution of weed seeds in clover seed sold in commerce, and this cannot be avoided unless and until improved means are found for cleaning seeds. Besides the seed sold within the law, which may contain noxious weed seeds, there is a considerable portion of the clover seed crop so badly contaminated with weed seeds that it is unmarketable to the regular seed trade and is a serious menace as a potential seed supply in the districts where grown."

By the Seeds Act of Canada, weed seeds are divided for each class of crop seed into four groups:

1. Prohibited noxious[1]
2. Primary noxious
3. Secondary noxious
4. Other weed seeds

according to the damage they do to the crops.

[1] Excellent coloured illustrations of the various weeds may be found in *Farm Weeds*, published by the Department of Agriculture, Ottawa.

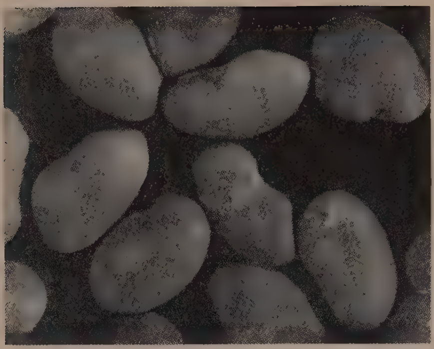

FIGURE 1. Alfalfa seed. Natural size $\frac{1}{20}$ inch approx.

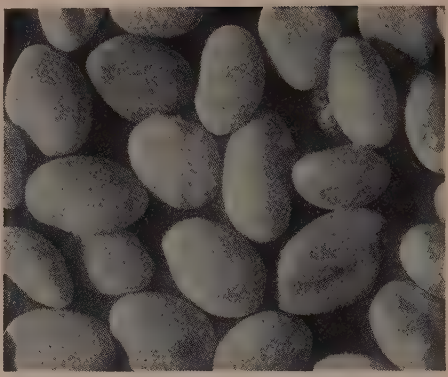

FIGURE 2. Sweet clover seed. Natural size $\frac{1}{20}$ inch approx.

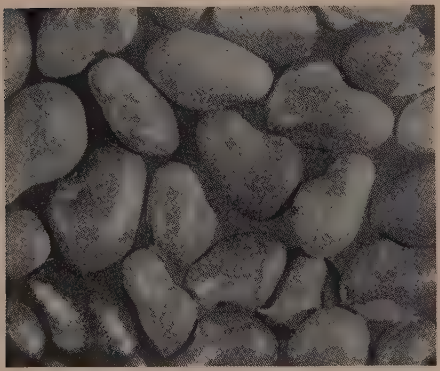

FIGURE 3. Red clover seed. Natural size $\frac{1}{20}$ inch approx.

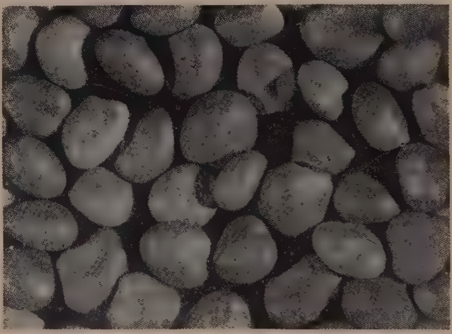

FIGURE 4. Alsike clover seed. Natural size $\frac{1}{30}$ inch approx.

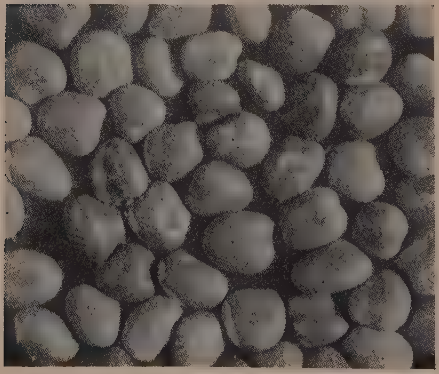

FIGURE 5. White clover seed. Natural size $\frac{1}{30}$ inch approx.

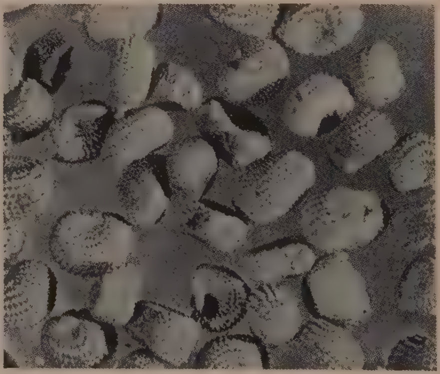

FIGURE 6. Bladder campion seed. Natural size $\frac{1}{20}$ inch approx.

FIGURE 7. White cockle seed. Natural size $\frac{1}{20}$ inch approx.

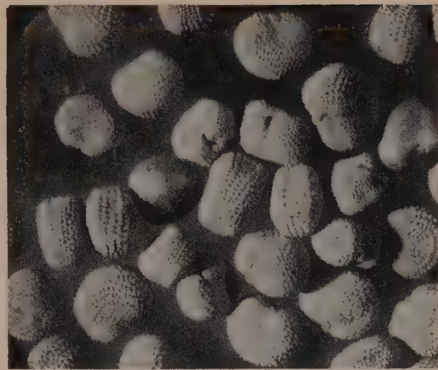

FIGURE 8. Nightflowering catchfly seed. Natural size $\frac{1}{20}$ inch approx.

For the seeds of alfalfa and clovers the prohibited noxious weed seeds include

Dodder (*Cuscuta* Spp.)

and five others.

Primary noxious weed seeds include

Bladder Campion (*Silene latifolia*)
White Cockle (*Lynchnis alba*)

and nine others.

Secondary noxious weeds include

Nightflowering Catchfly (*Silene noctiflora*)
Ribgrass (*Plantaga lanceolata*)
Field Pepper Grass (*Lepidium campestre*)

and 20 others.

The group of "other weed seeds" comprises all other species which are not in the above three classes nor are crop seeds and some of which, such as lamb's quarters and foxtail are difficult to remove from clover seed.

The seeds of bladder campion, white cockle and nightflowering catchfly are so much alike that it requires an expert to distinguish between them. (Figures 6, 7 and 8.)

The standard methods of separation from clover seed of the weed seeds named above leave much to be desired. The treatment of the campion-catchfly-cockle group of seeds is so difficult that no satisfactory method of separation was known, so that this group was selected for first attention. The difficulty in separating these seeds from the clover seeds arises from the fact that the weed seeds and the clover seeds are alike in being practically of the same size, the same shape, and the same specific gravity.

So many attempts have been made and have failed to give the desired separation it may be well to state how close the separation must be before the clover seed may be graded under the Seeds Act[2].

The Dominion Seeds Act specifies that for the clovers to make No. 1 grade there must be no prohibited noxious nor primary noxious weed

[2] The Seeds Act. Acts, Orders and Regulations No. 24, Published by the Department of Agriculture, Ottawa, Canada.

seed and not over five secondary noxious weed seeds per ounce in red clover seed, sweet clover seed, and alfalfa seed and not over ten per ounce for alsike and white clover seed.

In sweet clover, red clover and alfalfa seed there are from 15,000 to 20,000 seeds per ounce; in alsike about 40,000 seeds per ounce. In rejected seed there may be from a few to many thousands of these noxious weed seeds per ounce and yet the extraction of these weed seeds must be so good that the remaining clover seed must be entirely free from prohibited and primary noxious weed seed to be Grade No. 1 and within 1/40 of 1% of freedom from secondary noxious weed seeds to make the same grade. This degree of separation is away beyond any ordinary separation of metallurgical products. This high degree of purity must be obtained without a serious loss of good seed, otherwise the economic value of any separation would be lost.

To complicate matters still further it must be recognized that materials to be treated are living seeds that have to be used by farmers and that, therefore, nothing must be done to spoil or lessen the germination of the seed, and further, the seed must not have a sticky feel nor a bad colour, or the farmers will not want to buy it. Also, nothing poisonous nor bad-tasting nor bad smelling can be used in making the separation.

SAMPLING AND ASSAYING

Early in the investigation it was found necessary to have a quantitative measure of the degree of separation of the weed seeds from the clover seeds. A quantitative measure involves sampling and subsequent analysis. The nature of the seeds precludes any method of analysis except visual examination and detailed counting of the weed seeds in a weighed quantity of clover seed. Commonly a ¼ oz. sample is used, but sometimes it is necessary to examine a much larger quantity. To us this counting of the seeds becomes very tedious, particularly so because each seed must be seen through a glass magnifiying 5 to 10 diameters to make identification easy, though professional analysts are able to do the counting and identification without magnification.

To assist in passing the seed under the magnifier, a small picking belt, hand driven was constructed. A small hopper to hold 2 or 3 ounces delivers to a belt through an adjustable gate a stream of seed 4 or 5 seeds wide and 1 seed deep. Supported on a post by a hinge is a large size "linen counter" magnifying glass. By means of a needle in a wooden handle, individual seeds can be flicked off the belt into a hopper for examination. Otherwise, the seed stream delivers into a small bin at the end of the belt, and the required seeds counted as they pass under the glass and the number recorded by tripping a Veeder Counter for each seed.

A photograph of an early form of the picking belt is shown in Figure 9. Occasionally samples were sent to Dr. Leggatt[3] for an official count and for identification of individual seeds. Although this picking belt speeded up the analysis and removed some of the drudgery, the tendency is still to cut down the size of the sample to be analysed. It is not advisable to make the sample any less than ½ of an ounce. With this quantity, the errors

[3] See Foreword.

may be great, due to the difficulties of sampling. In counts that come near the border line of the various grades, it may be necessary to count several ounces. For sampling the seeds, a small Jones sampler was used by passing a small stream of seeds over the partitions of the sampler. No better method of sampling is known.

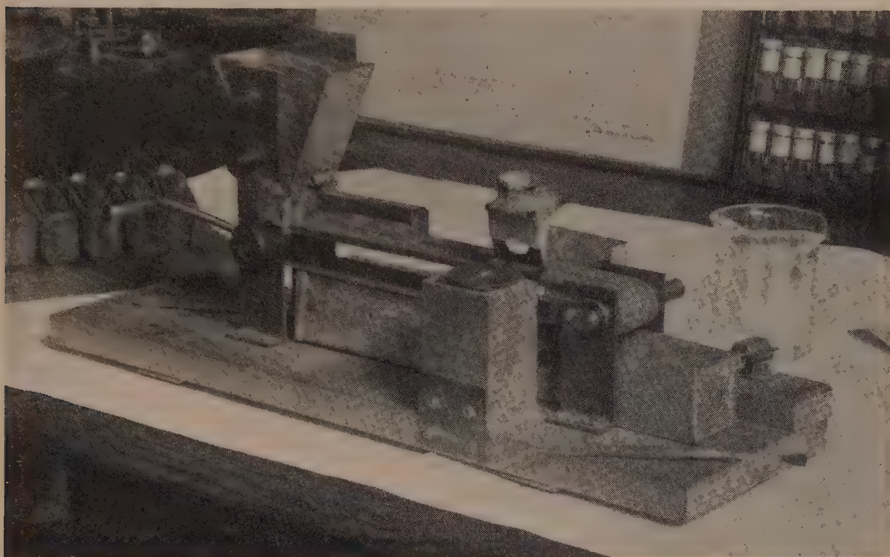

FIGURE 9. Counting belt.

As an illustration of the difficulty of sampling, consider these figures. A lot of sweet clover seed containing catchfly seed was divided by the sampler into 8 parts of $\frac{1}{8}$ ounce each and each part analysed with these results:

2 4 1 3 5 4 2 3 weed seeds.

Per ounce this is:

16 32 8 24 40 32 16 24 weed seeds,

an average of 24 weed seeds per ounce. A count of another ounce gave 22 per ounce. These figures show a standard deviation from the mean of 21.6%, and the resulting grade might have been No. 2, No. 3, or rejects, depending on which small lot had been taken for analysis.

Suppose it is stipulated that the tolerance in assay shall not be more than $\pm 10\%$ and that the degree of certainty shall be 80%, that is, the error will not be greater than 10% in 4 assays out of 5. Reference to tables of probabilities, such as, Tables II and III in Pearson's Tables for Statisticians, Part 1, shows that, with as many as 30 noxious weed seeds in the sample, to ensure that the certainty be 80%, it would be necessary to allow a count of 25 to 35, a tolerance of over 16%, much greater than the stipulated error. If the sample, even with this degree of error, was on the border-line between grade 2 and grade 3, that is, with 5 primary noxious weed seeds per ounce, it would be necessary, in order to come

within the permissible tolerance, to count about 6 ounces of seed, much too large a quantity.

With this grade of seed, and to ensure a degree of certainty of 4 times out of 5 on a count of 2 ounces, it would be necessary to allow the count to be between 7 and 14, a tolerance of 35%. With one-ounce samples, which are customarily the largest used, either the degree of certainty would have to be reduced or the tolerance increased.

In drawing conclusions from our assays this difficulty in sampling has been kept in mind, and before reliance was placed on differences in results of comparative tests the assays were scrutinized to see if the differences lay within the probable sampling error.

A further discussion of the sampling errors in seed analysis may be found in *The application of statistical methods to seed testing*, by G. N. Collin published as Circular No. 79, by the United States Department of Agriculture, Washington, D.C.

STANDARD METHODS

The standard methods of cleaning seed take advantage of differences in the size, shape, or specific gravity of the seeds. When any differences are present in any appreciable degree, simple and obvious methods may be used. The difficulty comes when the seeds to be separated approach each other closely in all of these characteristics. The natural procedure in a case of this kind where separations are difficult is first to test the usual methods of separation under delicate adjustment.

The literature dealing with the cleaning of clover seed is scanty. It seems that most seed cleaners are reticent on any development they may have made on the obvious processes. We found nothing that would suggest other than that the art was restricted to screening, air classification, water classification, with occasional use of heavy solutions, gravity separations on tables, and a few special processes to take advantage of outstanding differences in surface.

Gravity Separation on Oscillating Tables

The Sutton-Steel and Steel pneumatic table is used to some extent in seed cleaning plants but fails to separate the campion-cockle group of seeds from the clover. Repeated attempts with an ordinary Wilfley table also gave no results.

Hydraulic Classification

Separation of the campion-catchfly-cockle seeds from the clover seeds by rising currents of water is unsatisfactory.

The specific gravity of the seeds being about 1.2, there is very little sinking power in the seeds to begin with, so that vertical eddy currents in the water must be kept at a minimum. Further, the seeds are so much alike that even with closely sized seeds there is very little possible margin of difference in the densities of the seeds on which to work. Under the most carefully controlled conditions in a tubular classifier the separations obtained were never good. In the classifier used for testing purposes the effect of vertical eddies was reduced to a minimum by introducing a swirling motion to the water. A stirring device was added to the feed tube of the classifier to ensure that air bubbles were eliminated and also to overcome

the tendency of the seeds to agglomerate. By these means, the seeds were each given a chance to rise or sink individually and solely, according to their specific gravity.

In no sample of clover seed tested by this method was it found possible to produce a clean clover seed free from catchfly. In a few cases it was found, however, that by closely sizing the seed by screening before submitting it to classification, a lighter product could be obtained consisting almost entirely of catchfly seed, but the remaining clover seed was not catchfly-free. Later, this result, although it did not give the desired separation, was found to be important as an auxiliary to other processes. The diagram shows the arrangement of the classifier. (Figure 10).

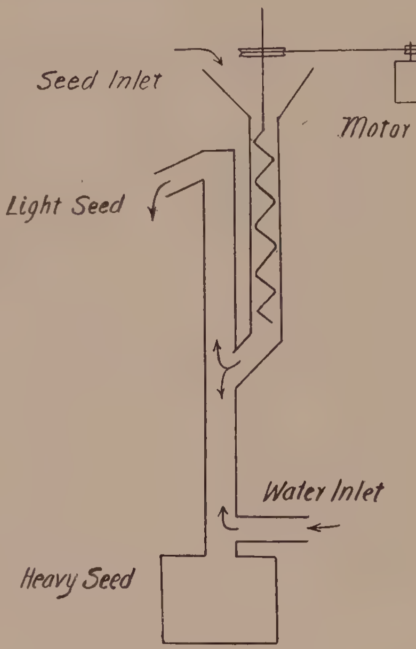

FIGURE 10.

The substitution of a current of air for the rising water currents was unsuccessful.

Sink and Float Tests and Centrifuging

"Sink and float" tests in salt solutions failed to make a separation. Eddy Seed Cleaners Ltd. report that salt solutions, even when reinforced by centrifugal action, only make a partial separation of the cockle group. The Eddy Company report was confirmed by small scale tests with a Babcock centrifugal cream tester.

None of the usual processes providing satisfactory separations even under the best of conditions, it became necessary to consider some unusual processes.

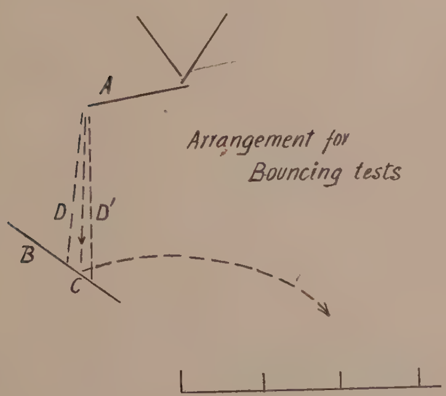

FIGURE 11.

Resiliency Tests

Remembering that sound cranberries can be separated from soft ones by making them bounce, trials were made to determine if this method could be applied to the seeds under investigation.

A simple piece of apparatus was set up (Figure 11) and the seeds allowed to fall from the edge of a feed chute (a) on to a glass plate (b) inclined at 45°. As seeds bounced from the plate they were caught in a series of parallel sample troughs. For the results of this

experiment to be definite, the seeds should fall along a simple line (c). It was found, however, that due, not only to small differences in size but also to the fact that a seed might pass over the edge on either its longer or shorter diameter, the seed would spread out and fall anywhere within the lines (d d′), and so the results were indeterminate.

The glass bouncing plate was replaced with a steel rule 1/16″ thick. Only the seed that bounced from this narrow edge was considered. The results were not promising and the experiments were discontinued. The differences in bounce of seeds of the same kind, depending on how they struck the plate, were great enough to mask the difference in bounce of two different kinds of seed.

Mushing Tests

It was discovered accidentally by leaving seeds under water over the noon hour that, when clover seed and catchfly seed were soaked in water, in the course of time, both absorbed water and became soft. The time required for the seed to soften varied with different lots of seed, and in the earlier samples tested the catchfly seed softened before the clover seed, or most of it did. It seemed from this that a time of soaking might be found which would soften the catchfly seed to a point where a fairly stiff brush would flatten out or mush the weed seed without damaging the clover seed. If this could be done, then a separation could be made by screening.

A number of experiments were made along these lines but the results were not satisfactory. The difference in the behaviour of various samples was very great. In some cases the weed seed softened first and in others the clover seed. Even in those cases where the weed seed did soften first some of the clover seed softened also, and some catchfly seed remained very hard. In any case the long soaking and subsequent expensive drying would only have been justified by a complete separation, of which this scheme gave no promise. The experiments were discontinued in spite of one case where a complete separation was effected.

Similar experiments were made by soaking the seed with various strengths of dilute hydrochloric acid and also of dilute caustic soda. There was no appreciable difference in results from those obtained with water except that many of the catchfly seeds turned red with the acid and the immature seeds seemed to soften a little faster than the mature seeds.

Differential Absorption

When clover seeds or weed seeds are wetted and allowed to dry slowly they will absorb the surface water and swell slightly. The amount of swelling will vary with the different seeds and with different lots of the same seed, and is probably a function of the amount of water absorbed. When the flotation process or the wet centrifugal process is used the time of contact with water is very short and since all excess water is promptly removed this swelling is very slight and the total moisture is well within what would damage the seed.

It has been observed that when just the correct amount of water was left with the seed in most cases a considerable proportion of the catchfly seed swelled more than did the clover seed after the seed had been in the bag for a day or two. This made it possible to separate some of the catchfly by screening provided the seed had been carefully sized in the first instance.

This method of separation did not give similar results with different lots of seed nor did it give the high percentage of separation required. Also it must be used with great exactness as too little water will not give the required swelling and too much may cause the clover seed to swell more than the catchfly seed, and also may cause the seed to become musty or to heat.

Air Friction Tests

There is considerable difference in the surface of the catchfly seed and the clover seed. The clover seed is smooth while the catchfly seed is "pimply" (Figure 8). It seemed possible that this difference might cause the seeds to be deflected differently if they fell freely into a very thin, horizontal, high velocity air jet. With very closely sized seed the pimply surfaced seed might be expected to gather horizontal speed due to air friction more quickly than would the smooth clover seed.

This was tried with a small orifice about 2 inches long and $\frac{1}{32}$-inch wide with $2\frac{1}{2}$ lbs. air pressure. The seeds were allowed to fall independently across this stream of air close to the orifice and were caught in a series of parallel sample troughs. The experiments were not conclusive.

The orifice was narrowed to 1/100 of an inch and the air pressure raised to 7 lbs. per sq. inch. The seed used was sweet clover containing much nightflowering catchfly seed. The resulting seed scatter was divided into three parts and analysed. For 10 gram lots the counts were: for the lot nearest to the orifice, 46 catchfly seeds; for the middle one, 54 catchfly seeds; and for the portion that was blown the farthest, 54 catchfly seeds. Although this difference is not more than a possible sampling error, it seemed to indicate that a higher air pressure might give better results and the air pressure was raised to 11 lbs. per sq. inch. This was sufficient to blow some of the seed 15 feet before it fell to the ground. The seed scatter was divided into 5 equal parts according to distance of travel, and analysed for weed seeds, giving the following counts:

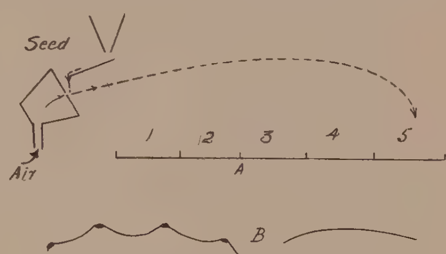

FIGURE 12.

Distance of travel in yards	1	2	3	4	5
Catchfly seeds per 10 grams	37	49	49	54	57

Although these results appear to justify the original assumption, there is no indication of any probability that a process of separation might be developed by this means to give the completeness of separation desired. Projecting the seed into the air from the pulley end of a fast moving belt gave similar incomplete separations.

Separation by Colour Differences

The recent great improvements in photo-electric cells have made separations possible by using "electric eyes" that were not thought of a few years ago. It is certain that a good degree of separation could be made of the campion-catchfly-cockle family of weed seeds from the clovers. It is desirable for a commercial machine to handle at least 4 bushels per hour. Since each seed must be examined individually and assuming that each electric eye can sort at the rate of 20 seeds per second, this would mean a machine containing over 500 electric eyes each with its accessory equipment. Such a machine, though possible, would be too costly.

Secondly, it is doubtful whether such a machine would operate to give the completeness of separation desired. Catchfly seed, which ranges in colour from black through brown to red, can be separated from sweet clover seed, which is yellow; but in the case of alsike clover, which contains a large proportion of very dark green seeds, a different set of "eyes" would be required, adjusted to shades of brown. We think the natural differences in colour of the same seed will prevent perfect separation.

Because of these colour variations and the high first cost of the machine required, we did not make any experiments to test the actual degree of separation possible. We do not consider this means of separation is practicable at present.

Electrostatic Separation

In testing this method a small electrostatic machine, giving between 30,000 and 40,000 volts was used. The materials tested were seeds air dried for several days, seeds stove dried, and seeds that were slightly moistened and then air dried until all surface moisture had evaporated. The seeds consisted of sweet clover with 50% bladder campion, sweet clover with 10% nightflowering catchfly, and alsike containing night-flowering catchfly. In no instance was there any noticeable separation. We do not think that for clover seeds this method holds any promise.

Magnetic Separation

The separation of one material from another by coating some of the particles with a magnetic substance and then removing the more heavily coated ones with an electro-magnet has in some cases been successful. This scheme was tried with sweet clover seed containing catchfly seed. The catchfly seed being rougher than the clover seed might be expected to hold more magnetic material than the smoother seed. High grade magnetite was ground until dusty in a ball mill and then mixed with the seed. The amounts of magnetite used varied from 1% to 7% of the weight of seed. The material was rolled in a bottle until every seed has a chance to become thoroughly coated. The coated seeds were passed through a Wetherill Magnetic Separator with a $\frac{1}{4}$ inch gap and amperages ranging from $1\frac{1}{4}$ to 15 amps.

The percentage of catchfly seed removed increased with increase of magnet strength but so did the amount of clover seed lifted (Table 1).

It was only with high amperage that an approximation to the desired removal of catchfly seed was obtained, and that at the cost of the loss of one-third of the original weight of good seed. Further, the good seed remaining was badly blackened with the magnetite, which was difficult

to remove even by washing with water. This dark seed is not marketable.

We have since learned of a process which uses magnetic separation after treating the seed with a magnetic material. The process is reported as being useful for some seeds, but does not appear to be in general use.

TABLE 1

Amperes	Portion lifted		Remainder
	Weight	Assay	Assay
	%	N.F.C.	per oz
$1\frac{1}{4}$	4.4	70	104
$4\frac{1}{4}$	14.7	50	24
$8\frac{1}{4}$	22.7	30	56
15	34.9	25	8

Flotation

Up to this stage no process tried had given any promise of success except possibly some form of vanning machine and even that did not hold out any great hope of success. Every other likely process had been tried except flotation, and without any expectation of success flotation experiments were made, and according to expectation they failed.

Both types of modern flotation machines were used in laboratory sizes. One type provides a voluminous, brittle froth, of large bubbles, with minimum agitation. The other type gives a comparatively small volume of a stiff froth, with small bubbles, and uses violent agitation.

Frothing agents used were pine oil, alcohols, soaps, "Snap", and an extract from the weed seeds themselves which has considerable frothing properties.

Collecting agents tried were linseed oil and some other organic oils, coal oil and other petroleum products.

The circuit was either acid with hydrochloric acid or made alkaline with lime, caustic soda or sodium silicate.

It can be readily realized that the choice of possible reagents is very limited because of the organic nature of the seeds and the subsequent use to which they must be put. Further, except in a most vague way, nothing is known of the character or surface composition of the seeds which might be a guide to the selection of reagents. Consequently every experiment was a pure and simple "shot in the dark". Later when more was known of the differences of seed coatings these flotation experiments were repeated and others made. The results were consistent failures in obtaining even a fair approach to a separation.

The reasons for the failure appear to be:

(1) A very small difference in wettability of the various seeds.

(2) Failure to find a suitable harmless reagent to increase this difference in wettability.

(3) The turbulence inherent in modern frothing flotation machines to a large extent prevents the attachment of bubbles to the seeds.

(4) The seeds have such a low specific gravity, so little greater than water, that they have a very small sinking power and are consequently mechanically trapped in the froth.

It is not impossible that with increasing knowledge a froth process might be modified to suit these seeds but at present the use of modern commercial flotation machines is not possible.

DEVELOPMENT OF A FLOTATION PROCESS

All the previously described experiments having proved unsuccessful, the only other way to attack the problem of the separation of clover seed from the catchfly group of seeds appeared to be to find some unknown property of one of or both the seeds that might lend itself to the object in view.

Nothing had been found in the literature that offered any lead, so a more or less random series of experiments were undertaken. One series consisted of soaking the seeds in any liquid that came to hand and watching the results under the microscope.

Having been divested of any pre-conceived notions and prejudices, no liquid that came handy was passed by without a trial; the liquids ranging from distilled water to Bass' Pale Ale and chloroform. There were no conspicuously unusual results. It so happened that one lunch hour some mixed seed had been left in water on the stage of the microscope and before an open window. During the rest period some of the water evaporated and left the seeds sticking up out of the water like islands, and dust that had blown in with the wind had settled on the water. This was nothing unusual, but what was new was that the floating dust accumulated around one kind of seed in preference to another. Because of those few specks of dust an investigation that was almost dead came to life. That dust showed there was a difference in the curvature of the water surface around the different seeds, therefore there was a difference of surface tension; therefore some kinds of seeds must be more wettable than other kinds, and therefore the flotation process, being dependent on differences of surface tension, ought to work as a means of making a separation of the various weed and other seeds. It had not worked when tried previously. Was the evidence of the dust a delusion? A resort to the soda water bottle would soon tell. Some sweet clover and catchfly seeds were sprinkled on the bottom of a beaker and covered with water. They all stayed on bottom. A squirt from the syphon introduced bubbles. When the first fizzing was over the remaining gas precipitated on the glass of the beaker and on some of the seeds. In a few minutes all of the clover seed had been raised by the bubbles to the surface of the water along with a few catchfly seeds, but most of the weed seeds remained at the bottom. Flotation as a means of separating clover seed from catchfly seed is a possibility in spite of the evidence from previous failures.

The previous experiments with modern flotation machines were repeated. Changes in method of operation, such as feeding the seed to the froth instead of to the pulp, and other variations were tried but the conclusions arrived at and stated previously were confirmed.

An approximate comparative measure of the wettability of some of the seeds was obtained by placing a number of each seed on blotting paper and then placing the paper on water. The paper soon became waterlogged and sank, leaving all the seed floating. This floating was not because the seeds were lighter than water for the specific gravity of all these seeds lies between 1.2 and 1.4. Each day the number of seeds that had sunk was counted (Table 2).

These numbers do not show any very marked differences except in the case of white cockle, which is distinctly more wettable than the others.

TABLE 2.—PERCENTAGE OF SEEDS THAT HAD SUNK AT THE END OF EACH DAY

No. of days	Bladder campion	Night f. catchfly	White cockle	Alfalfa	Alsike clover	Sweet clover
1st	0	1	10	0	0	0
2nd	1	3	10	1	0	0
3rd	3	4	39	1	0 S	0 S
4th	3	4	40	50	60	40 S
5th	3 S	4 S	42 S	95 S	90	95 S
6th	5 S	4 S	55 S			

S—Means that the floating seeds had begun to sprout.

All these seeds must have a water resistant skin, otherwise they would have absorbed water and sunk. The three clovers show a slightly greater resistance to wetting than do the three weed seeds. These figures cannot be used as absolute measures of the degree of wettability of the various seeds for the seeds absorb water in varying degrees and each kind of seed varies considerably in itself from one lot to another, but they do show that the bladder campion group of seeds has outside coats that are more easily wetted than the outside coats of the Clover group of seeds.

A method more susceptible to adjustment than the modern flotation processes was required. The film flotation process seemed to be the most promising, and this method was developed finally into a commercial means of freeing clover seed from the seed of bladder campion, nightflowering catchfly and white cockle. Subsequently greater knowledge added dodder and others to this list.

Preliminary trials were made on a test tube scale. These trials developed into a standard routine method for testing the feasibility of proposed working conditions which later could be tried on a larger scale. A measured quantity of seed, about a third of a test tube full, is placed in a tube and water or other liquid added till the test tube is half full. It is then shaken by hand for a period of ½ minute. The seeds are dumped onto a piece of 30 mesh wire cloth, and spread by the finger to a layer one seed deep. The bottom of the screen is then wiped with a towel to remove excess moisture and the seed allowed to dry for one minute. The piece of screen at an angle of 15° from the horizontal is then introduced edgewise into a basin of still water, allowing some seeds to sink and to be removed with the screen. The floating seeds are gathered by pouring the water onto another piece of screen. The two lots of seed are then washed through a funnel into a long narrow graduated glass tube closed at the bottom by another piece of screen onto which the seeds are finally washed for drying or immediate counting. The relative quantities are read from the graduated tube. Although this method cannot give very precise results, it is very useful for comparative tests and is a great time and labour saver when hundreds of tests are required.

The fundamental principle of this method is: (1) to wet thoroughly all the seeds; (2) expose the wetted seed to air so that some of the seeds may dry more quickly than others; (3) present the seeds individually to the surface of a water bath; (4) the seeds which have dried will float on the water by surface tension while the seeds that are still wet will sink.

Among the many factors which affect the operation of this method, the principle ones are: (1) the nature of the seed coat; (2) the thoroughness of the wetting; (3) the presence of substances which help or hinder the wetting; (4) the degree of agitation while wetting; (5) the time allowed for wetting; (6) the time allowed for drying; (7) the speed at which the seeds reach the surface of the water bath; (8) the angle of entry to the water; (9) the surface tension of the water; and (10) the temperature of the water.

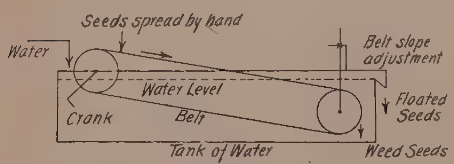

FIGURE 13.

The first machine used to test the operation of this method on seeds was simply a piece of rubber belting travelling around two rollers and dipping into a tub of water (Figure 13). This device developed into a continuous operating machine with a rubber belt 12 inches wide dipping twice into water and provided with a stirrer to mix the seed water and oils, etc., with feeding and measuring devices, etc. (Figure 14). A canvas belt was tried on this machine and discarded. The rubber belt was not satisfactory, partly on account of the interference of waves washing up on the belt, and on account of the difficulty of removing the water during the drying period.

The next machine used a wire cloth belt around three rollers (Figure 15) and gave such promising results that a larger experimental machine

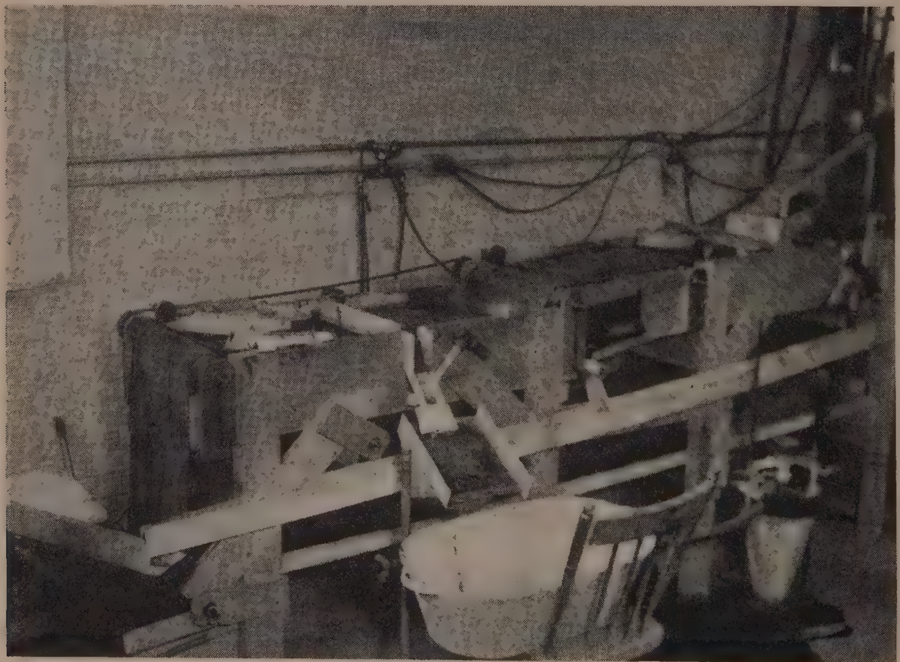

FIGURE 14

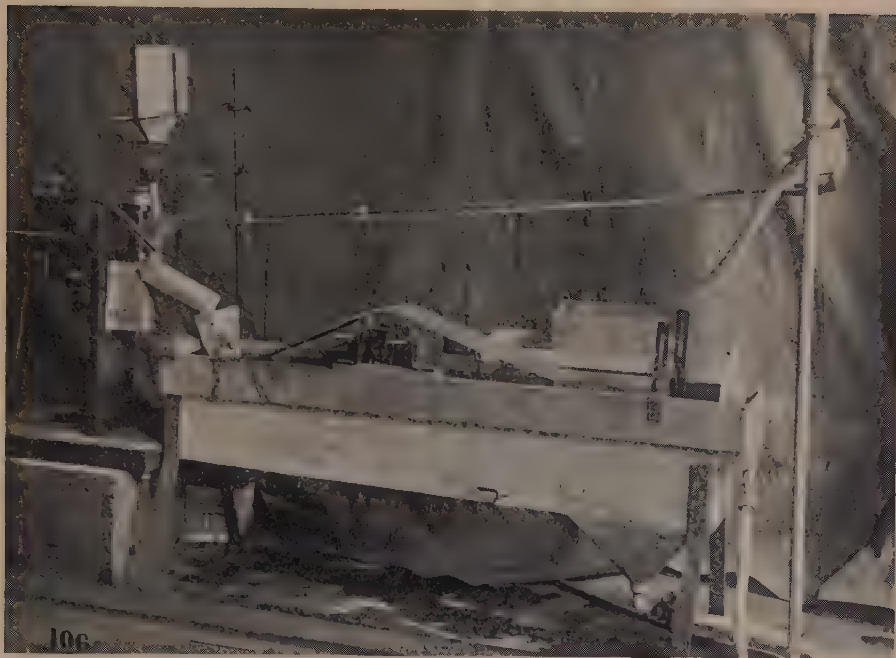

FIGURE 15.

FIGURE 16.

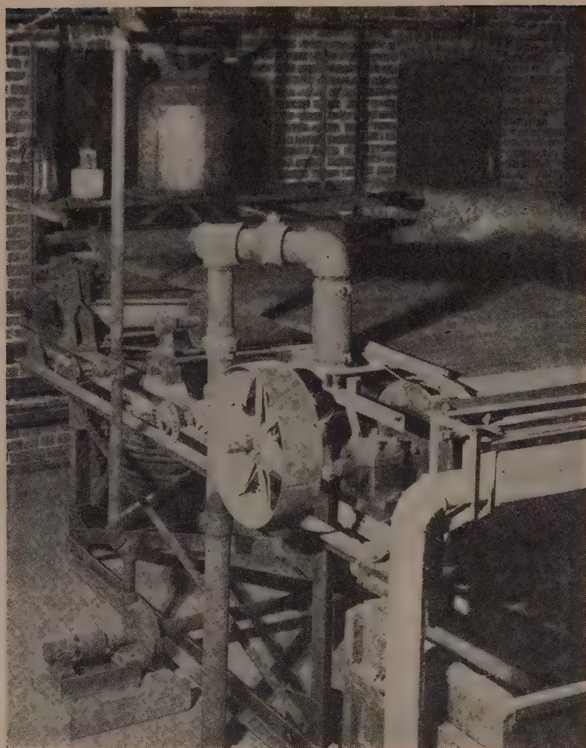

FIGURE 17.

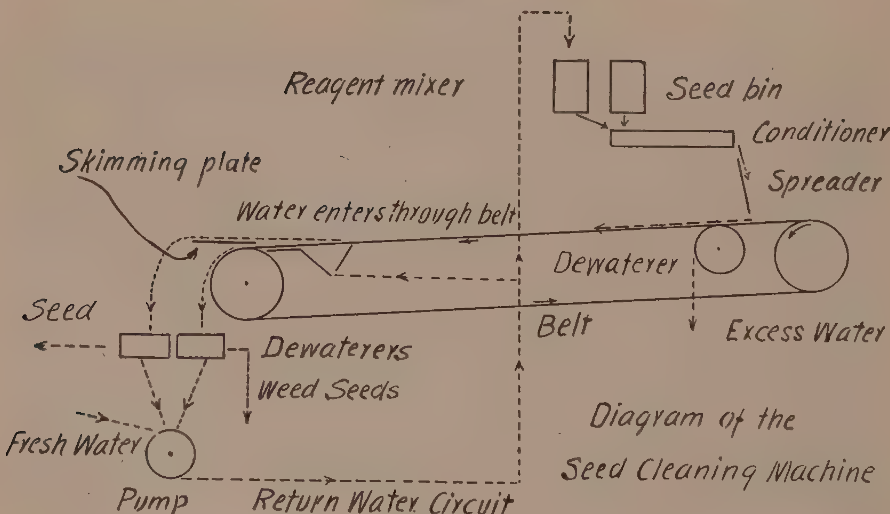

FIGURE 18.

(Figure 16) was placed in the plant of a professional seed cleaner and was operated by the ordinary plant staff. The machine was not operated continuously and chiefly at night, but in the two years it has been in use, it has treated over a million pounds of seed, made up of many small lots and has proved that the process is commercially practicable.

Operating this machine revealed many mechanical weaknesses, and suggested technical improvements which led to the building of a larger machine (Figure 17). The general scheme is shown by Figure 18, the most notable feature of which is the elimination of the large tank of water used in the earlier machine. This change made possible or necessary other changes, of which some of the details are given in the following sections.

Conditioning

This term denotes the preliminary preparation of the seed by wetting with water; or by wetting with water containing a wetting agent to increase the wetting properties of the liquid; or by water in which an oil or other substance has been mixed to decrease the wetting of the clover seed. With most lots of the seed under consideration, wetting with ordinary water is sufficient.

The early conditioning machine consisted of a screw conveyor in a pipe into which the seed and water were admitted. This type was abandoned because the seed flow depended on the water flow rather than on the screw. If the flow slowed up by reason of too little water, or too much seed, the swelling of the seed blocked the conditioner. A feed screw in an open trough gave better results but would give a steady discharge only with a thick feed. The flotation results using this conditioner were erratic and it was abandoned. Tests with these conditioners showed there was something lacking in the completeness of the wetting of the campion and catchfly seed. Immersion in water for a considerable time, up to several minutes, was not always sufficient. It was found that to wet these two seeds, bladder campion and nightflowering catchfly, properly, a period of violent agitation was required. To provide this agitation, the screw of the conditioner was replaced by a shaft equipped with many paddles and revolving at a fairly high speed. It was necessary to depend on the conditioning water to effect transport through this machine. This conditioner worked fairly well; subsequently other experiments showed that so long as the agitation was sufficiently violent for a very short time, any subsequent time of immersion was of no advantage so far as the weed seeds were concerned and only made the clover seed more difficult to float. Given a sufficient degree of agitation, the maximum time required for wetting campion and catchfly seeds is not over 30 seconds and usually is much less.

The present conditioner is a cylinder very short compared to its diameter. It is about $2\frac{1}{2}$ inches long and 9 inches in diameter. The seeds and water enter and leave by central openings at the ends of the cylinder. In the cylinder is a revolving disc on which are pins which pass concentrically between similar pins on the inside end of the

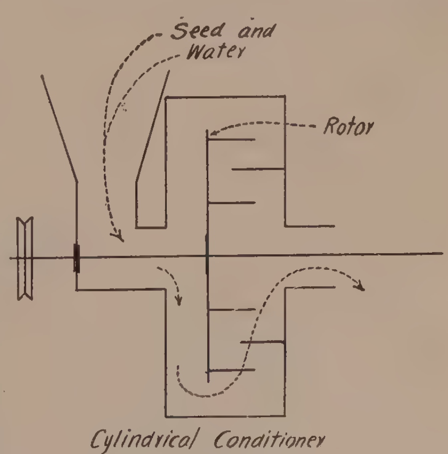

FIGURE 19.

cylinder, at 600 to 800 R.P.M. This conditioner (Figure 19) gives all the agitation necessary. It is easily cleaned, has small storage capacity so there is little delay in the passage of the seeds, and consequently the absorption of water by the seeds is a minimum. There is sufficient clearance in the machine so that the seeds do not become broken nor damaged so that their germination is detrimentally affected.

Effect of Agitation in the Conditioner on Germination

Growing tests on alfalfa and alsike seed after agitation for periods up to 16 minutes did not show a variation in the percentage germination beyond the probable sampling error.

The reason for the necessity of agitation in the wetting by water of the seeds of campion and catchfly can be understood by reference to the photographs of these seeds. The extreme tip of the tubercles is quite different in texture from the hollows. The tips are hard and difficult to wet. The hollows are easy to wet but apparently a film of air is to some extent trapped in the hollows so that the film of water on the wetted seed is not held very firmly, and so the seed easily becomes dry in spots. The specific gravity of the seed is so little greater than that of water that in the case of bladder campion seed, a dry spot covering three or four tubercles may be enough to cause the seed to float with the clover seeds. The violent agitation presumably drives out the air film.

Conditioning Agents

For wetting the seeds of bladder campion, nightflowering catchfly and white cockle, plain water is usually effective when coupled with sufficient agitation. In some lots of seed, the clover becomes wetted too easily and various oils are used to coat the clover seed with a more water resistant surface. The oil is previously beaten up with water to help distribution before adding it to the conditioner. Excess of oil tends to float the weed seeds also, particularly if the seeds have not been previously thoroughly wetted. Several oils were tried. A moderately light oil such as neutral white petroleum was found to be suitable when an oil was necessary. Other oils might have been used except that they failed in some of the requirements such as tastelessness, or had a tendency to rancidity, or stickiness on standing, or had a disagreeable smell. It is better to have no smell at all. The use of an oil did not always prevent an excessive loss of clover seed through too much wetting. Some of the reasons for this loss are discussed later.

Distribution of the Seed on the Belt

In the original larger scale machine, the seed was delivered to the belt under water which made it easy to spread the seed only one grain deep by means of a straight edged scraper. In the later machine without the big tank when the seed was delivered to the belt, the water ran through the belt leaving the seed in heaps. A straight edge scraper spreads the seed one grain deep but trouble was found in keeping the seeds from climbing up on the delivery side of the scraper and then falling off in clots two or three seeds deep on the belt. The objection to these bunches of seed is that the lower clover seeds may be forced under the surface of the water

and be lost with the waste or that weed seed might be rafted on the floating clover seed and be carried into the good seed with the possibility of lowering the grade of the treated clover seed. This bunching was prevented by inserting a strip of wire cloth $\frac{1}{2}''$ wide into the scraper about $1''$ above the lower edge of the scraper. This allowed a thin film of water to pass through to the delivery side of the scraper, thereby preventing the seeds from being held by surface tension at the junction of the water surface and the dry side of the scraper (Figure 20).

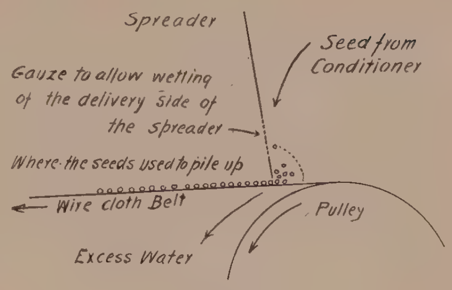

FIGURE 20.

Drying the Seed on the Belt

Most of the water is removed almost as soon as the seed reaches the belt. Part of the water is carried by surface tension in the meshes of the belt and some more as a film over the seeds.

The ideal condition is to have a water film around the weed seeds and none around the clover seed. With the openings of the belt full of water, it takes a long time for the film to dry or to break over the clover seed, so it is desirable to remove any excess water as fast as possible, thereby diminishing the length of belt necessary to give the clover seed a chance to finish drying (Figure 21).

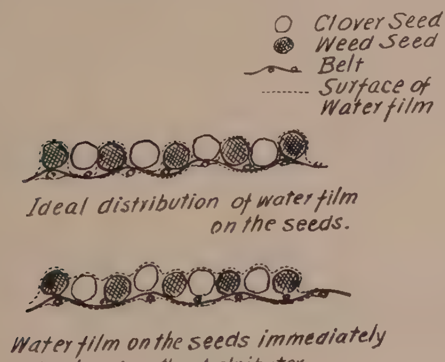

FIGURE 21.

A roller covered with sponge rubber and pressing on the underside of the belt was found more efficient than a vacuum in sucking the water from the seed and the belt. A squeegee roller under the suction roller keeps it from becoming saturated. It is not desirable that the suction roller be quite dry. The suction roller is more positive than a vacuum or than air pressure in removing the excess liquid as it uses the capillarity of the fine pores of the rubber to overcome the surface tension of the water film on the seeds, and is independent of the amount of seed on the belt.

The Separating Bath

The moment at which the seeds reach the water surface of the separating bath is the critical moment during which the seeds part company, the dry ones floating on the surface of the water and the wet ones continuing their way under the water.

To avoid a sudden shock to the seeds from a quick change of speed the bath water is pumped up through the belt which at this point is nearly

horizontal. The travelling belt drags the surface water along with it so that belt and water are travelling at approximately the same speed and with only a small angle between the belt and the water surface. It seems to be important that the change of momentum and the change of direction of travel of the seeds at the point of introduction to the separating water should be a minimum. This way of presenting the seed to the water surface makes it possible to run the belt at high speed, the limit being governed by the time taken for the clover seed on the belt to become dry. The material of the wire cloth belt must be easily wetted. If the belt is not easily wetted, the change of direction from belt to water surface will be abrupt because of the surface tension curve. This water curve coupled with high speed belt travel causes a water wall over which the clover seeds cannot climb, and therefore are forced under with the waste seed (Figure 22).

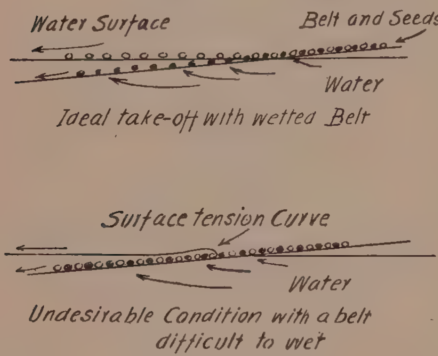

FIGURE 22.

To reduce the tendency of the water to form this undesirable wall, it is preferable to use a wire cloth of comparatively open weave and thoroughly degreased. We have found that a 32 $\times$ 32 mesh monel metal ventilator cloth is suitable. When put into service new it requires to be degreased like other wire cloths. After being in use for some time the monel metal takes on a dark coating probably from reaction with some of the products derived from the seed and it then is much more easily wetted than before.

When the seed is once divided into two layers, "floats" and "sinks", it is easy by means of a horizontal splitting edge just below the surface of the water to conduct these layers into different channels for de-watering the seed.

Drying the Seed after Flotation

The flotation process being one in which the seeds are wetted, the question arises as to whether the seed will absorb enough water in the few seconds during which the seed is under water to affect its keeping qualities.

In practice in a moderate climate, such as Ontario, it has been found that neither the keeping qualities nor the germination deteriorates because of the wetting, provided the seed is de-watered without delay by centrifuging. The centrifuge leaves some surface moisture on the seed which is absorbed in a short time by the seed. Several tests have shown that the total moisture after treatment is about 11% and sometimes less. In the case of wheat the moisture content may be over 14% before the wheat is classed as tough. Some small lots of clover seed with 16 to 18% moisture have been kept in sealers for months without becoming musty, so that around 11% moisture appears to give a safe margin. The cleaned seed may be put into the usual grain sacks without any special drying, and so

far has given no trouble either by heating or going musty provided the bags are stored so that the air can circulate between them. In a warm climate, or if the seed is to go into storage in a large bulk, it might be necessary to pass the seed through a dryer. Also, clover seed dried only by a centrifugal after flotation will lose some moisture and weight under ordinary warehouse storage conditions. If the seed is to be weighed and bagged for market directly after flotation, it should first be dried to normal to avoid subsequent loss in weight.

Some of the Variables in the Operation of the Process

Original Moisture in Seed

One of the differences noted between various lots of seed is the moisture content, which varies with the length of time since the seed was threshed and with the conditions of atmospheric moisture in the place where the seed may have been stored for a length of time. If the seed has been through a cleaning process this also may affect the moisture content. The moisture content would possibly vary the flotability of the seed. Flotation tests show that this is so.

The moisture content of the seeds was determined by loss in weight after drying the seed for three hours at a temperature of 110° C. After adding water and giving time for the water to become distributed, the moisture content again was determined by drying.

To fresh samples of sweet clover of originally 9.4% moisture, enough water was added to raise the moisture content, and after being bottled for three days to distribute the moisture evenly, the seeds were tested by flotation (Table 3).

TABLE 3

Lot No.	Percentage moisture in the seed	Percentage of seed floated	Nightflowering catchfly seed per oz. in float
1	10.3	92.5	364
2	11.6	95.5	428
3	15.4	94.0	652
4	17.2	85.0	1160
5	22.9	83.0	1790

The percentage floated is a maximum at about 11% moisture. In No. 4, the sweet clover was slightly musty which accounts for the large amount which sank. In No. 5, the seed was appreciably swelled. An unexpected result was the steady increase in the amount of catchfly seed floated, which quantity increased with the amount of moisture in the seed.

A further test was made with alsike to check the effect of absorption of moisture from the air. The alsike seed had been stored in a warm dry place for 2 years and then placed in a humidifier to allow the seed to absorb water (Table 4).

TABLE 4

Time in humidifier in hours	Float		Sink	
	Per cent	Assay	Per cent	Assay
		per oz		%
None	93.6	22	6.1	50
45 hours	90.72	26	9.28	40
90 hours	96.5	144	3.5	70

The assay is for nightflowering catchfly seed.

In this case as before the catchfly seed floated the better for extra moisture, but so did the alsike. A more definite connection between moisture content and flotability is shown in the next test.

Several 100-gram samples of old alsike were placed in sealers, varying amounts of water were added, and the sealers were closed for 18 days. The samples were then put through the flotation machine. The results are shown in Table 5.

TABLE 5

Water added to 100 gr. seed	Total percentage of moisture	Percentage of alsike sunk
None	7.2 (dry seed)	7.64
0.25 cc.	7.4	6.1
0.75 cc.	7.88	6.77
2.5 cc.	9.4	7.71
5 cc.	11.5	8.55
10.25 cc.	15.62	17.6
15 cc.	18.8	22.0
20 cc.	22.7	76.0 (This lot became musty)

Thus we see that the minimum sink occurs when the moisture content is somewhere near normal. Increase in moisture content over the normal amount increases the percentage sunk, although the seed appeared to be perfectly dry.

Surface Impurities such as Soil, Road Dust, Etc.

One lot of alfalfa seed when treated by flotation on the large machine in the ordinary way gave a loss by sinking of 25% of the seed. The seed was dull in appearance and showed at 10 magnifications a very few small particles of clay sticking to the surface of the clover seed, though none could be seen by the naked eye.

Laboratory tests confirmed the heavy loss. Longer conditioning time with the idea of removing the clay gave results still worse than before, the loss rising to 30%. It was noted that the conditioning water became slightly dirty, so a portion of the seed was placed in a jar and agitated briskly for a couple of minutes with water. The water was decanted and the seeds treated immediately by flotation with a resulting loss of only 10%. The water was filtered and the mud examined microscopically. Some quartz particles were seen and many limestone particles, as well as clay, organic matter, etc. The origin of the seed was not known but it apparently had been grown in a field near a country road from which the dust had been blown by the wind over the clover. In the process of threshing which removed the hulls from the clover the road dust had evidently dirtied the seed. After thorough washing to remove the dirt this seed could then be easily cleaned.

The Effect of Common Salt

There are some processes of seed cleaning that use a gravity separation aided by a heavy liquid, usually common salt solution. The presence of common salt is detrimental to subsequent cleaning of the seed by flotation methods.

Several samples of alsike were received from the Eddy Seed Cleaners, Limited, who reported that they were unable to clean the seed because of a difficulty in floating the seed. From 65 to 70% of the seed would

not float. A check test in the laboratory confirmed the result, 60% being non-flotable.

The past history of the seed indicated the probability that the seed had at some time been treated with a salt solution and that the residual salt in the seed was the cause of the trouble.

The original seed as well as samples of sink and float as produced by the factory were analysed for salt (Table 6).

TABLE 6

Seed	Lot No.	Product	Salt present
			%
Alsike	S.B.B.	Original feed to flotation machine	0.39
Alsike	S.B.B.	Float from flotation machine	0.024
Alsike	S.B.B.	Sink from flotation machine	0.045
Alsike	Lot X	Check sample from another lot	None

Common salt was found where there normally is none. The reduction in salt content in the "float" and "sink" is due to the washing of the seed while passing through the machine.

Several attempts were made to thoroughly wash the salt from the alsike and then make a separation. Also, it being possible that the washing would have allowed the seed to soak up enough moisture to spoil flotation, the seed was dried in various ways after washing and before flotation (Table 7).

TABLE 7

No.	Seed	Lot	Procedure	Total seed sunk, %
1	Original feed	Alsike S.B.B.	Washed salt free, treated on machine while still wet. No oil or conditioning agent used.	43.5
2	Original feed	Alsike S.B.B.	Washed and seed dried quickly on a hot-plate. No oil.	45
3	Original feed	Alsike S.B.B.	Ditto but with slower belt speed to help flotation.	35
4	Original feed	Alsike S.B.B.	Like No. 3, but seed dried 3 days in air before flotation.	45
5	Check	Alsike LX	Check with salt free seed. Conditions as in Exp. 1.	10 (5% Alsike) (5% weed)

Washing until the washings were sufficiently salt free to give no reaction with silver nitrate did not restore the original condition.

The supply of seed, alsike lot S.B.B. being exhausted, tests were continued by salting other fresh seed. The seed in weighed amounts was immersed in concentrated salt solution, whirled in a Babcock centrifugal machine for various times to ensure thorough wetting, drained, washed and floated (Table 8).

Table 8.—Effect on flotation of time of contact of seed with common salt solution

Time of contact of seed with solution of salt	Percentage of total seed sunk on flotation after drying			
	Very quickly on hot plate	3 hours on hot plate	3 days without heat in air	No drying. Fed wet to machine
1 minute	39	22	74.1	10.7
2 minutes	44.2	—	74.8	14.9
3 minutes	51.9	—	82.7	20.9

In each case longer contact with salt solution caused a larger percentage of the clover seed to become unflotable.

That this loss is not due to the absorption of moisture by the seed during soaking can be seen by reference to the tests on the effect of moisture where after two days in the humidifier the loss on flotation was less than 10%.

From these tests it is evident that treating the seed with salt is detrimental to flotation; that the detriment is greater with increase in time of contact with salt; that once the seed is sufficiently salted washing will not restore the flotability; and no amount of drying will bring the flotability back to its original amount. Also, that if the time of treatment with salt is sufficiently short the amount of sinks may still be within commercial limits.

Heated Conditioning

It being known that temperature has a marked effect on surface tension the result of applying heat to the conditioning water and to the drying seed on the belt was tried. Heating the conditioning water might be expected to work in two ways, by helping to disperse the conditioning agent if any is used, and by hot water having a greater wetting effect than cold water. The application of heat to the seed on the belt would hasten, perhaps preferentially, the drying of the various seeds.

The tests were made on a sweet clover and the results applied later with marked success to a large shipment of a refractory alsike seed. Subsequent tests show that the application of heat to the conditioning water is not always advantageous.

The sweet clover was, to begin with, difficult to float as shown by experiment 1 of the following table. When floated with no conditioning reagent and the conditioning temperature at 10° C., 49% of the seed sank compared to only 0.8% sinking under identical conditions except that the temperature was raised to 75° C. A similar marked change occurred where treating an alsike seed with hot conditioning water containing oil. A change in temperature of the conditioning water from 12° C. to 75° C. decreased the percentage of seed sunk from 24.5% to 2.5%.

A series of tests using various methods of applying heat to the seed, such as heating the conditioning water, or heating the seed with steam or hot air while on the drying belt, showed that heat may be desirable and does not damage the seed during the short time of exposure if the temperature of the seed does not rise above 80° C.

From results obtained in experiments on sweet clover seed containing 22 campion seeds per ounce, we found that the application of heat, either by heating the conditioning water or steaming the belt, is very beneficial with some lots of seed in the separation of campion seed from sweet clover seed, whether a flotation reagent is used or not. Similar results were obtained when alsike seed containing 125 catchfly seeds per ounce received heat treatment. Under normal conditions over 60% of the alsike seed was unfloatable. The reagent used was a 1 to 1 carbon tetra chloride petroleum mixture concentration of 1 part in 400 of water (Table 9).

The increase of temperature in conditioning reduced the loss of alsike seed from almost 68% to less than 2%, the cleaned seed containing less than 5 nightflowering catchfly seed per ounce, the grade being raised from "rejected" seed to grade No. 1. However, raised temperatures were not always advantageous. On some lots of seed it made no particular difference to the separations. In other cases, while the heat treatment aided the flotation of the clover seed, it also hindered the sinking of the weed seeds spoiling the concentration. It was found also, that the easiest method of heat treatment was by heating the conditioning water, and it was also more effective, particularly when a flotation reagent was used.

Table 9

Temperature of conditioning water		Sunk, %	Float assay compion
10° C.	*No reagent*	68.0	6
10° C.	Reagent used	18.4	5
45° C.	Reagent used	6.5	10
75° C.	Reagent used	2.48	6
85° C.	Reagent used	1.63	3

The tests in Table 10 show that the undesirable effect that may follow heated conditioning. The seed was Canadian red clover screenings containing about 7000 dodder per ounce, the reagent water-glass, 1/600, and citronella was used in the flotation bath.

Table 10

Temp.	
15° C.	41.4% sunk, 4800 dodder per oz. in float.
50° C.	30.0% sunk, 7200 dodder per oz. in float. All the dodder seed floated.
85° C.	Only about 0.1% sunk. Practically all the seeds floated.

To summarize briefly, we have found heated conditioning sometimes beneficial in the separation of campion and catchfly seeds from sweet clover and alsike seeds. Why each kind of seed does not always react the same way to heat treatment is not known. The surmise that the heat aided the distribution of the oil used as a conditioning agent may be true, but it is not the prime cause.

Fouling of Water Circuit by Seed Extracts

When clover seed and some of its associated seeds are scaked in water several materials are dissolved out of the seed by the water. In course

of time these substances accumulate in the water circuit of the flotation machine unless considerable fresh water is continually added. These extracts have a decided effect on flotation. From sweet clover seed is extracted cutin and coumarin, and from alsike a green colouring matter; Bladder campion and nightflowering catchfly seeds are a source of saponin.

These substances may act by reducing the surface tension of the water at the separation end or by increasing the wettability of the seed at the conditioning end. In either case, the floating of the seed is hindered with increased losses.

The effect seemed to be more pronounced with sweet clover seed than with other varieties. Tests were made with sweet clover seed to determine the seriousness of this contamination. Some of the results obtained, using seed extracts as conditioning agents were as follows. Weighed quantities of seed were immersed in 5 litres of water for 1 minute at 85° C. The resultant extract was diluted to 5 litres and used in the conditioning circuit, with the temperature maintained at 85° C., and the separating bath temperature 10° C. The seed was mixed sweet clover containing 450 catchfly and campion seeds per ounce.

The result of the seed extracts accumulating and fouling the separation water is to increase the percentage of clover seed floated and at the same time to increase at a greater rate the percentage of the weed seeds floated. As the fouling became greater, the percentage of clover seed floated became less, with the weed seeds declining in flotability at a slower rate. The net result is that the substances dissolved from the seeds, while being conditioned, caused the grade of the floated seed to become poorer. The curves (Figure 23) show the reversal which suggests that there may be

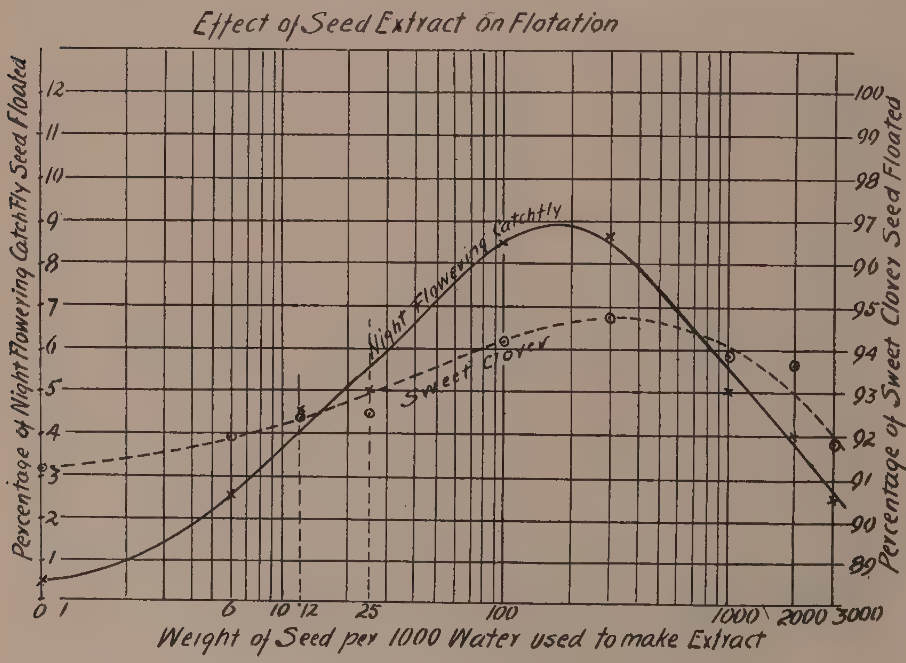

FIGURE 23.

more than one substance dissolved from the seed and which act in different concentrations. These substances must lie close to the surface of the seed in order to be extracted in the short time the seed is wet during the flotation process. In the case of sweet clover, the colouring matter seems to be the first to dissolve, followed by coumarin, which gives sweet clover its characteristic smell. Staining tests followed by microscopic examination of sections show that coumarin lies almost entirely in the layer of Malpighian cells, the second row from the surface, and therefore is easily extracted. The campion and catchfly seeds are sources of saponin, which is a decided reducer of flotability. The coumarin in solution increased in quantity up to test 6 and then was approximately constant.

Polishing and Flotation

In the flotation of clover seed, particularly sweet clover seed, it frequently happened that a small percentage of the seed stubbornly refused to float, even though the bulk of the seed gave no trouble. An attempt was made to find a reason therefor.

It was noticed that seed that had gone through a scarifier had a tendency to be more difficult to float than unscarified seed. This suggested that in the passage through the scarifier the seed surface was rubbed or abraded. To produce a surface change on the seed to a maximum degree on small samples, a polisher was constructed following the general lines of a polisher in the plant of the Eddy Seed Cleaners. The photographs show the construction of the machine (Figures 24 and 25). The

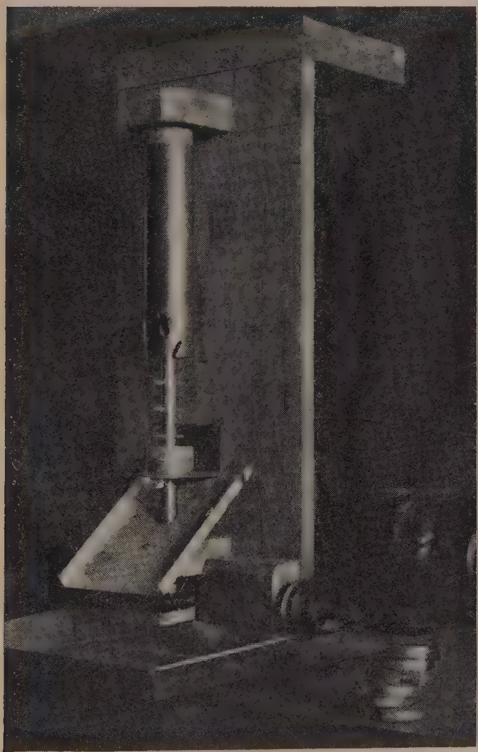

FIGURE 24.

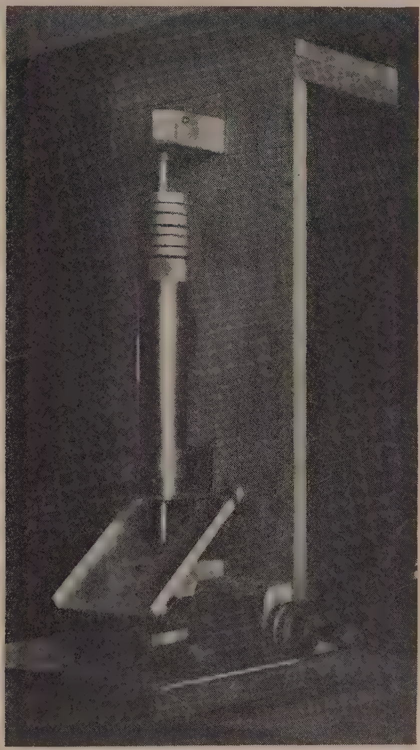

FIGURE 25.

weights shown are equivalent to the weight of a column of seed 4 feet high. The revolutions of the spindle are sufficiently slow (125 R.P.M.) as not to produce any breaking or deformation of the seed. Because some lots of seed are originally very dirty, covered with gritty road dust, some lots of seed were polished after mixing with sand, some after mixing with very small chilled shot (−48 mesh), and others without the addition of anything.

During the course of polishing a dark powder is produced, presumably the outer coat of the seed. This dust is easily removed from the seed by dry wiping or by washing with water. On removal of the dust the seed seems as bright as the original seed (Figure 26). There is no apparent change so far as visual examination is effective. That there is a change is shown by the marked decrease in the flotability of the polished seeds.

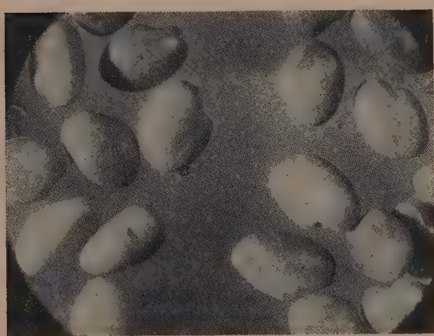

FIGURE 26.

Not polished. Sweet clover seed. Dry polished.

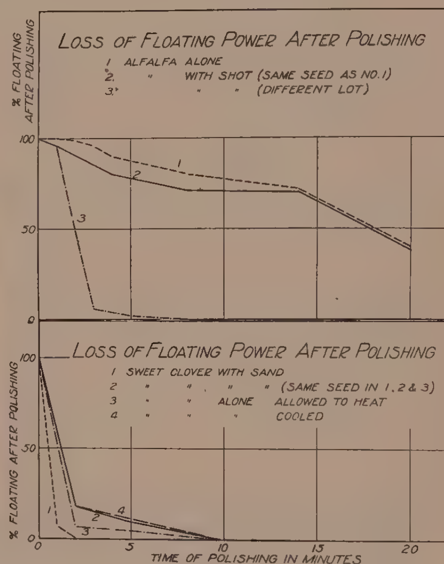

FIGURE 27.

The curves of Figure 27 give an idea of the extent of these changes. The figures from which the curves are drawn are only approximations because of the errors of sampling and because of small changes in technique. The results, however, show very decided trends along the following lines.

(1) Rubbing the clover seed changes or damages the outside layer.

(2) The floating power decreases with increase of rubbing or polishing.

(3) Raise of temperature while polishing increases the rate at which flotation decreases.

(4) The presence of grit increases the rate at which flotation decreases after polishing.

(5) With the same kind of seed, different lots vary in the effect of polishing.

(6) The average loss of floating power due to polishing of one kind of clover seed may be quite different to the similar loss of another kind of clover seed.

These results give one explanation of why the clover seed, in particular sweet clover seed, may be at times difficult to float. The sweet

clover seed coat appears to be more delicate than the coat of other clover seeds and therefore suffers more in threshing and also in scarification for the treatment of hard seeds. Sweet clover seed more than other clover seed may require scarification to increase germination. Subsequent experience on commercial lots of seed confirms the detrimental effect of polishing by friction on the flotation of clover seeds.

Another commercial method of polishing seed is by giving the seed a slight coating of paraffin oil, not enough to make the seed sticky nor feel oily, but just enough to remove the dull appearance of the seed. The seeds are dry at the time of mixing with the oil, therefore the weed seeds as well as the clover seeds become oiled, removing the natural differences of wettability and making separation by the flotation process impossible.

For example, a lot of yellow blossom sweet clover seed containing 85 bladder campion and catchfly seeds per ounce was treated in the usual way with a resulting loss of 35% of the clover seed. When conditioned with oil and the conditioning temperature raised to 50° C., the loss in the sinks was still 30%. Testing this seed in the laboratory under various conditions of temperature and with different reagents showed that the difficulty lay with the seed and not with the operation. The smallest loss of seed obtained was 9%. In all the tests of this seed the loss was very high. The best results were obtained by using a considerable amount of oil to float the clover seed and adding a wetting agent to sink the weeds. Heating the conditioning water above 50° was of little or no advantage. A moderate increase of temperature aided by improving the distribution of the heavier oil. A longer drying time than usual reduced the percentage loss but at the expense of the grade of the float. This seed was very dull in appearance, though washing with water gave no signs of earth or dirt. Filtering the wash water and microscopical examination of the solids showed considerable cuticle that had been removed from the seed. Judging by the results of friction polishing this seed had received rough treatment, possibly by being threshed before it was properly cured; consequently it was difficult to float.

Germination after Polishing

Some lots of clover seed after being floated and dried are improved in appearance by polishing. Advantage was taken of some tests on the effect of polishing by friction on the flotation of clover seeds to see what difference this mechanical polishing would have on the germination of the polished seed.

Some lots of red clover seed that had been polished were tested for the proportion of hard seeds after various times of polishing. The curves of Figure 28 give the results. With this seed there is an early reduction in the number of hard seeds while long continued polishing makes little further reduction.

The germination of clover seeds depends on the penetration of water into the seed at the region called the strophiole[4]. Hamly shows that in a hard seed a small blow in the region of the strophiole is sufficient to allow water to enter and permit germination. He shows that severity of impact is not necessary. Scarification by breaking the seed coat also allows water to penetrate the seed but causes many damaged or weak seedlings.

[4] Softening of the seeds of *Melilotus alba*. Douglas H. Hamly, Botanical Gazette—Vol. XCIII—1932.

If the same softening can be obtained by putting momentary pressures on the strophiole the hardness could be reduced without damaging the seed. This the polisher appears to begin to do, but not to the extent that might be expected.

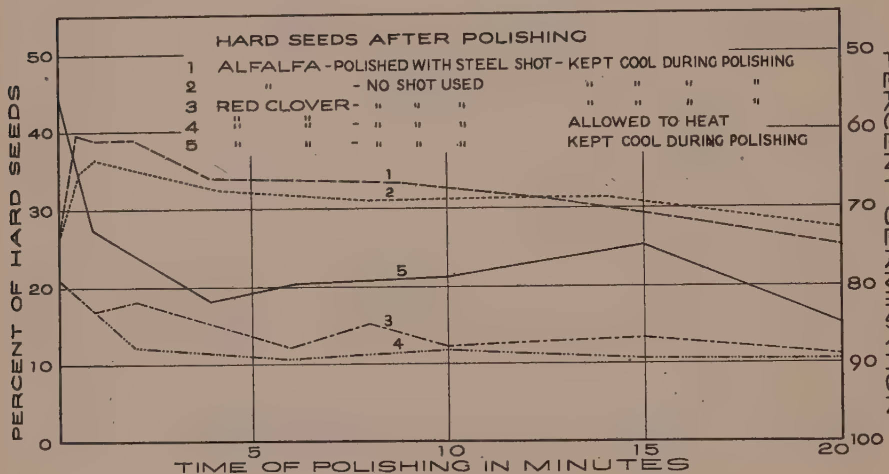

FIGURE 28.

It is assumed that the incompleteness of the softening is possibly due to the fact that the strophioles are to a considerable extent protected from being pressed by the other seeds because of being somewhat in the hollow of the clover seed (Figure 29). If the seed be mixed with smaller particles that can enter this hollow to squeeze or impact the strophiole, then more complete softening might be expected from the use of the polisher.

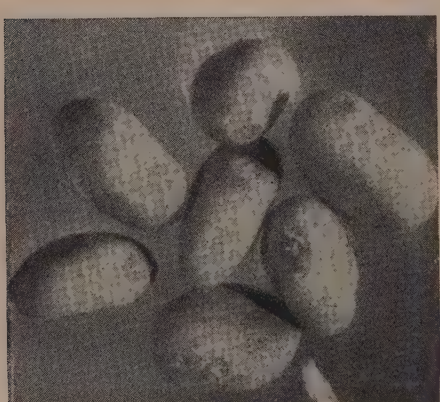
FIGURE 29.

Germination tests were therefore made on a sample of sweet clover seed that had been mixed with an equal volume of well rounded sand size 14/28 mesh and then put through the polisher. Counting the hard seed brought out the unexpected fact that instead of a decrease there was an increase in the number of hard seeds for the first minute or two of polishing, and then a decrease to about the original percentage (Figure 30, curves 1 and 2). The decreased hardness shown by curve 3 is what might be expected from the statements of other workers on the effect of heat on the hardness of seeds[5], that a moderate raise in temperature aids the germination of clover seeds. In one test on alfalfa (Figure 28, curves 1 and 2) very small chilled cast iron shot (35/65 mesh) was used instead of sand with similar results.

[5] Ewart, Miller, Harrington, Lute. (See Hamly's bibliography.)

From these results it may be judged that the effect of the polisher was in the first place to plug the strophioles in some way thus increasing the hardness. This effect is more apparent on some lots of seed than on others though whether the difference is inherent in the different kinds of seeds or only depends on which lot of seed was used is not known. However, this shows that the mechanical polisher must be used with discretion.

Curves 1 and 2, Figure 28, and curves 1, 2 and 3 of Figure 30, the curves of alfalfa and sweet clover seeds, each indicate an increase in the number of hard seeds in the first minute or two of polishing. Curves 3, 4 and 5, Figure 28 of red clover, show a decrease in the number of hard seeds in the first minute of polishing. No attempt was made to determine if the difference between the red clover seeds and the other seeds was specific or was due to some peculiarity in the conduct of the experiments.

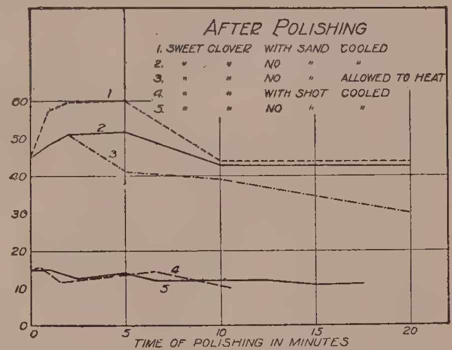

FIGURE 30. Hard seeds

This increase in the hardness of seeds produced by the polisher is unfortunate for it was proposed to use the polisher to make clover seed unfloatable in the separation of it from some hard coated seeds on which the polishing has little effect.

During the polishing, the cuticle was removed without making any great difference in germination properties while having at the same time a marked effect on the surface wettability. Flotation and germination are therefore totally unrelated phenomena.

A Quick Test for Hard Seeds of Sweet Clover

The usual method of determining the hardness of clover seed, particularly of sweet clover, is to make a germination test of the seeds by keeping them moist between blotting papers in lots of 100 or 200 and counting the number that fail to sprout or show distinct signs of swelling. For clover seed the count is made at the end of three and of five days. For the seeds to swell, water must penetrate the seed coat either through the strophiole or through breaks in the seed coat. Seeds that show no signs of swelling after 5 days are considered to be hard seeds. A more rapid test may be used where hardness and viability may be considered to be synonymous or in new seed where the life of the seed may be taken for granted.

If the seeds are soaked for a few minutes with a weak solution of ferrous sulphate or other iron salt and then dried in air, the outer coat remains colorless. If the solution can penetrate to the inside of the seed the ferrous sulphate in a few hours will turn the inner seed coat jet black, so that a black spot on the seed indicates a place where water can soak in and therefore black spots on a seed indicate that it is not a hard seed. Under this test hard seeds retain completely their natural colour.

Many checks made of this method against the ordinary germination method all showed that differences if any were within the probable sampling error.

This is a ready test for hardness in light coloured clover seed when the hardness and not the total viability of the seed is required.

Natural Variations and Flotability

Experiments have shown that some of the causes of the difficulty in floating all the sweet clover seed are: (1) the result of polishing or rubbing in the process of threshing or some subsequent treatment; (2) the presence of dust and dirt on the seed, probably road dust from fields near the highways; (3) a third reason was found in a few lots to be the presence of common salt retained from a previous operation; (4) another cause of unknown origin is the one that can be removed by heat treatment. These reasons do not altogether explain the reluctance of a portion of the sweet clover seed to float. It was thought that there might be some chemical difference between the varieties and strains of the sweet clover, which might appear in the reactions of the seed.

Yellow and White Blossom Clovers

Tests on lots of pure white blossom and pure yellow blossom seed showed that one variety contained no more coumarin than the other, and the flotability of the two kinds was about the same.

Bitter and Sweet Strains of Sweet Clover

There are two strains of sweet clover, a bitter strain and a sweet strain. This bitterness is associated with the presence of coumarin. It was thought possible that there might be some association between the bitter and sweet strains and the flotability of the seed. When J. S. Clayton and Professor Larmour[6] developed a quick method for the determination of coumarin, tests were made on various seeds to see if flotability was connected with coumarin and possibly other connected chemical differences.

In tests on several pairs of sinks and floats we find that the float in many cases contained more coumarin than the sink, but not always, indicating that the chemical properties associated with coumarin were not the cause of persistent sinking.

Hardness and Flotability

The tests on polished seed show that the induced hardness of sweet clover seed due to polishing by friction is accompanied by loss of floating power. It does not follow that the original hardness is connected in any way with flotability. Tests for hardness on the sink and float of a clover seed known to contain considerable hard seed showed that there is no direct relationship between the natural hardness of the seeds and their flotability.

THE SEPARATION OF DODDER SEED FROM CLOVER SEED

There are several species of dodder of which it is difficult to differentiate the seeds, so a rough classification is made into large seeded and small seeded dodders according to whether or not the seed will pass a screen of one millimeter opening. The small dodder seeds can usually

[6] A comparative color test for coumarin and melilotic acid in *Melilotus* species. J. S. Clayton and R. K. Larmour. Canadian Journal of Research, C. 13 : 89-100. 1935.

be removed from clover seed by screening. The seed of the large seeded dodders presents more difficulty, yet it is essential that dodder be absent from good seed, for both in Canada and the United States of America and elsewhere it is classed as one of the worst weed seeds to be found in clover.

All of the known methods of separation of dodder from clover seed depend on the minute roughnesses of the dodder seed coat, Figure 31. In one method the depressions in the seed coat are filled with a specially prepared magnetic material so that the seed can be lifted with a high powered magnet. In another process the slight differences between the rough dodder seed and the smooth clover seed are used to produce by friction a differential throw of the seeds when allowed to fall onto a fast moving surface faced with velvet. Another method feeds the seed to a trough formed by two rolls covered with fabric and rotating in opposite directions at a comparatively slow speed. This by repeated treatments brings about a progressive improvement in the separation to the point where a clean product can be obtained. These methods tend to give considerable middling product. Another disadvantage is the small capacity.

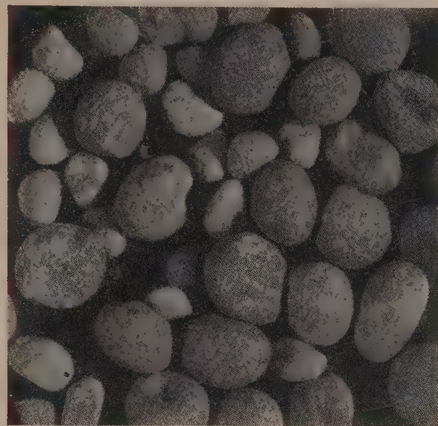

FIGURE 31. Large and small dodder seeds.

The authors considered it desirable to find another method that might give better results and one that would remove dodder seed at the same time as bladder campion, cockle and similar seeds.

The flotation process proved successful in the case of bladder campion and night-flowering catchfly seeds, so an attempt was made to apply the same principles to the separation of dodder seeds from clover seeds.

The simple wetting and flotation process that served for night-flowering catchfly did not prove as successful when applied to dodder. Just as with the campion-catchfly-cockle seeds, vigorous agitation was found to be necessary in wetting the dodder seed and probably for the same reason. Agitation was therefore made a part of the standard procedure similar to the method outlined previously. Most of the tests were made on a test tube scale, any tests that showed promise being repeated on a larger lot of seed.

Of the chemical nature of the cuticle of the clovers very little is published[7] and even in that little the authorities do not always agree. About the outside of the dodder seed still less is known so that the only fact with a bearing on flotation on which to build a system of experiments is that the dodder seeds like the nightflowering catchfly seeds, but to a smaller degree, are more easily wetted by water than are the clover seeds.

[7] A. M. Lute. Impermeable seed of alfalfa. Bull. 326, Colorado Exp. Station.

D. H. Hamly. Softening of the seeds of *Melilotus alba*. Bot. Gazette, Vol. XCIII, 4. 1932.

A. M. Anderson. Preliminary michrochemical study of the seed coats of Grimm Alfalfa and Red clover. Bot. Soc. of Am. 1930.

The tests were made along the following lines:

(1) To increase the difficulty of wetting the clover seed so that the dodder seed might be made to sink.

(2) To decrease the surface tension of the water bath.

(3) To use a reagent to increase the wettability of the dodder seed compared to the clover seeds.

(4) To increase the specific gravity of the dodder seeds.

Previous tests had shown that it is possible to decrease the wettability of clover seed by the use of paraffin or similar oil. However, paraffin instead of increasing the difference of wettability between dodder seed and clover seed increased the flotability of the dodder seed so that in some tests the differential of wettability between the seeds disappeared and both seeds floated together.

Decreasing the surface tension of the bath aided dodder to sink more readily, but also, though not quite to the same degree, helped the clover seed to sink. The lowered surface tension of the bath by itself was not sufficient to make a good separation possible though as will be seen later controlling the surface tension of the bath may be advantageous when used in conjunction with other factors.

Raising the temperature of the conditioning water improved the separation to some extent but still a considerable portion of the dodder seed persistently floated. With a sample of red clover containing about 500 dodder seed per oz., using a conditioning temperature of 75° C., and a belt speed such that 1.8% of the seed was sunk there remained 180 dodder seed per oz., in the float. When the belt speed was increased so that 6% of the feed was sunk there still remained 118 dodder seed per oz. in the float. Raising the temperature of treatment by itself was not enough to ensure a separation.

Flotation tests were made on Canadian red clover, Canadian red clover screenings, and on American red clover screenings. Tests were made on each seed with the conditioning both hot and cold, with water glass as a depressant, with petroleum as floating agent, with citronella in the separating bath to reduce its surface tension, and several combinations of these, without approaching nearly to the degree of separation required. Some other factor was evidently required.

To use a conditioning agent in order to increase the wettability of the dodder seed to a greater degree than that of the clover seed seemed to be the most promising method. As a slight guide to the choice of reagents to be tested the reagents at first were grouped as those that would chemically attack or dissolve (a) cellulose, (b) fat, (c) lignin. Since the Maule colour test did not give any reaction with the dodder seed coat, and those reagents such as chlorine water, potassium permanganate, which attack lignin, did not aid the separation, it is assumed that lignin is absent from the seed coats.

Not all of the reagents and combinations tried are mentioned below; only a few out of the sixty odd to indicate the trail followed.

Of the cellulose group, zinc chloride and caustic soda were of no benefit. Schweitzer's reagent (copper dissolved in ammonia) made possible a fair separation. The reagent stained the dodder seed but not the clover seed.

Although the reagent for chemical and economic reasons would not be suitable in practice it confirmed the possibility of there being some other reagent that would increase the differential flotability between the clover and dodder seeds by making the dodder seed the more wettable.

Carbon tetrachloride, a fat solvent, allowed some separation but acted mainly as an oil in reducing the wettability of the seeds. Trichlorethylene made no particular difference. The oxidizing agents, sodium peroxide and chlorine water were of no advantage, though hydrogen peroxide gave a slightly greater differential in the wettability of the two classes of seeds.

Another line of attack was to attempt to precipitate an easily wetted substance preferentially onto the dodder seed. Most of the tests gave negative results. A few showed a possibility. Precipitating cellulose from solution in Schweitzer's reagent by acid in presence of the seeds allowed a fair separation by flotation to be made. Treating the seed with a wash of barium chloride followed by sodium sulphate improved the separation, though both barium chloride and sodium sulphate were classed as being of no use when either was used alone.

Boiled wheat starch though effective in the treatment of some silicates proved to be useless, as did starch hydrolyzed by caustic soda. Starch hydrolyzed by hydrochloric acid permitted a better separation than was possible without any reagent in the water of the conditioner.

Still following the basic idea of anchoring a film of water around the dodder seed more firmly than around the clover seed, the seeds were rolled in powdered calcium chloride in the expectation that the slightly rougher dodder seed coat would take up the powder more readily than the smooth clover seed. By passing the seed dusted with calcium chloride through a fog made from cooled steam the chloride being very hygroscopic would take up water and therefore the dodder seed should become wetter than the clover seed and it might be expected that the dodder seed would thereby be helped to sink in the separating bath more readily than the clover seed. What actually happened was that both seeds, the dodder as well as the clover, became more difficult to sink and every seed floated. No attempt was made to find the reason for this surprising result.

In the laundering and dyeing industries great advances have been made in the use of chemical detergents and wetting agents as replacements for soap. Some of them such as the sodium silicates are well known, others are of recent origin and new ones are still coming on the market. Samples of many of these were obtained and tested.[8]

Of the sodium silicates, water glass S. brand gave the best results, giving a cleaner float and a smaller sink than the bi- and meta-silicates. Probably this is due to the higher alkalinity of their solutions which would also account for the greater discoloration of the clover seed by the bi-silicate and meta-silicate than when using water glass as a conditioner.

Most of the detergents were quickly eliminated, some on account of the large quantities required to make any appreciable change, others on account of negligible results up to the point of solubility of the material in water and similar reasons. The most promising ones were those sold under the trade names of the Gardinols, Dupolols, Avirols. Chemically

[8] The sodium silicates were from E. T. Sterne & Co., Brantford, and other washing compounds were obtained through the courtesy of Mr. H. Eastwood of Canadian Industries Limited.

these are sodium alcohol sulphates differing according to the particular fat used in their manufacture.

Small quantities of the mixed seeds were wetted with solutions of the reagents up to 10% strength or up to the limit of their solubility in hot water. The wetted seeds in each test were spread on a piece of gauze, the excess moisture removed by wiping the underside of the gauge with a towel, and then introducing the seeds to the surface of water. The number of dodder seeds and of clover seeds floating were then counted and the percentage plotted. While these results may not be as exact as if made on large samples, the results were sufficiently good to be made a basis for eliminating the less suitable reagents. The most promising of these reagents are the Gardinols which gave a larger differential in the sinking of dodder and clover seed with a smaller quantity of reagent than the other materials tried.

The Gardinols

Each of them wets the dodder seed more completely than the clover seed. The wetting properties increase with the strength of the solution, but there are marked differences between their differential wetting properties as shown by Table 11 and the curves of Figure 32.

With increasing strength of solution the differential wetting becomes less. Since the object is to obtain the greatest degree of wetting of the dodder seed and at the same time the greatest differential, the results with Gardinol WA and solution strength from 1% to 2% appear the most promising.

TABLE 11

1% solution of	Gardinol WA.	Gardinol CA.	Gardinol LS.
Clover floated, %	55	75	60
Dodder floated, %	10	22	50
Differential	45	54	10
2% Solutions			
Clover floated, %	33	40	43
Dodder floated, %	4	10	22
Differential	29	30	21

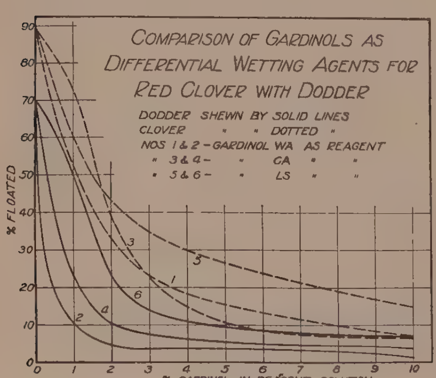

FIGURE 32.

In wetting cotton goods Gardinol CA has proved more efficient than Gardinol WA in hot solutions, but in parallel tests to those cited above Gardinol CA invariably gave poorer results than Gardinol WA and consequently was discarded with the other reagents.

Gardinol WA seems promising provided the percentage of clover seed wetted can be reduced without losing the wetting effect on the dodder seed.

The Use of Oil

Previous work has shown that paraffin oil is adsorbed by clover seed making it more difficult to wet, but dodder appears to have the similar characteristics as is shown by first treating the seed with Gardinol and then by various oil water emulsions.

Oil Water Emulsion, Following Gardinol WA

Parts of water to 1 of oil	500	1000	2000	4000	12,500	40,000
Clover seed floated, %	94	91	89	85	80	60
Dodder seed floated, %	90	87	87	70	22	10

Oil, Water and Gardinol Emulsified Together

Treating the seed with 1 oil in 20,000 water emulsified by G. WA increased the differential but floated more dodder.

Oil Water Emulsion Followed by Gardinol

Treating the seed with oil-water emulsion first (1 oil in 20,000) and then by Gardinol WA 2% sol. increased the differential, but still 16% of the dodder seed floated. The use of paraffin oil apparently requires careful control if used at all and tends to float dodder in spite of the depressing effect of the Gardinol (Figure 33).

The Effect of Heat

Heating the conditioning water has in some cases been found beneficial. Tests were made with 1% and with 2% Gardinol solutions, no oil, and

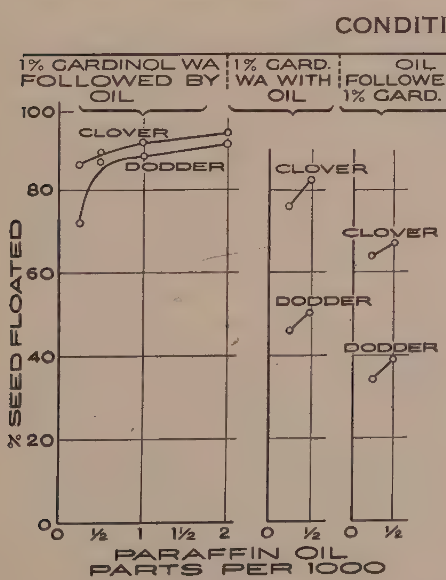

FIGURE 33.

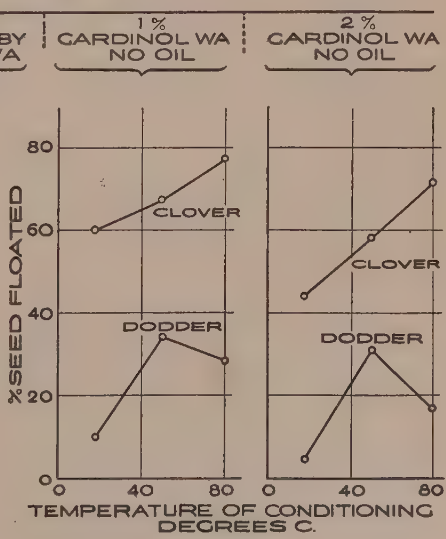

FIGURE 34.

CONDITIONING BY

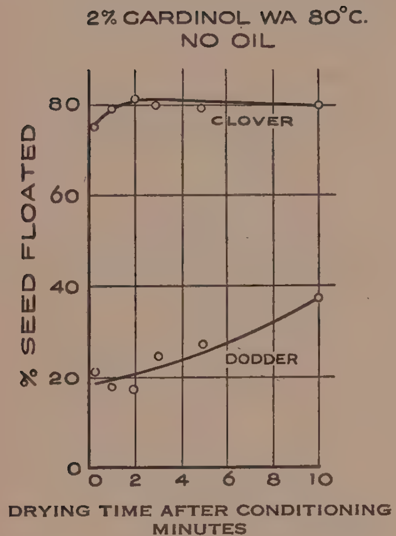

FIGURE 35.

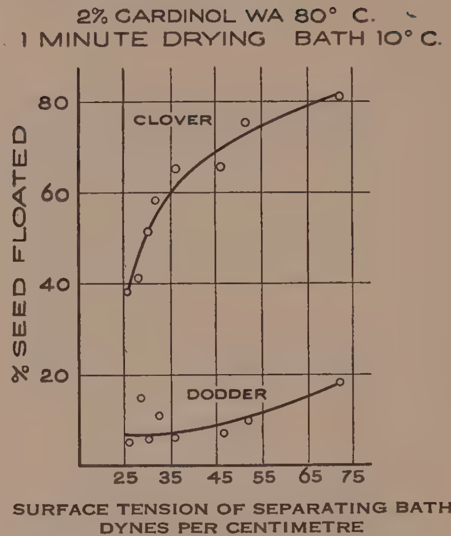

FIGURE 36.

at 50° C. and 80° C. The best result was with 2% Sol. and 80° C. giving a differential of 54% with 16% dodder floating. This is somewhat better than with cold reagent. The addition of an oil treatment gave a poorer result, a differential of 50% with 18% of the dodder floating (Figure 34).

Drying Time

Another factor that may affect the differential between the floating properties of clover and dodder seeds is the time allowed for the seed to dry after conditioning.

In the previous tests the best result was a differential of 54% obtained by using 2% Gardinol solution at 80° C. without oil. These conditions were maintained and the drying time increased. The tests showed that the percentage of clover seed floated remained remarkably constant with drying times varying from 1 min. to 10 min., (Figure 35). Evidently the clover seed which does dry sufficiently to float does so very quickly. The percentage of dodder seed floating decreases during the first 2 minutes possibly due to the absorbtion of water, after which the flotability increases, probably due to drying.

Surface Tension of the Bath

The actual separation of the seeds from each other is a function of the surface tension of the bath. A weaker S. T. would allow a larger proportion of the seeds to sink. The S. T. may be reduced by heating the water. This did not prove satisfactory. A better way is by the addition of a contaminant in which connection Gardinol is very effective. The surface tension figures corresponding to the amount of Gardinol in solution were obtained from the C.I.L. booklet. As might be expected a drop in the amount of both clover seed and dodder seed occurred, the drop in the case of clover being the greater (Figure 36).

Using citronella as a S. T. reducing agent gave practically the same results, a differential of 67% with 9% of the dodder floating.

Washing after Conditioning with Gardinol destroyed the wetting effect of the Gardinol on the dodder, the percentage of dodder seed floating rising from 5–10% to 60–70%.

Increased Conditioning Time might be expected to decrease the percentage of dodder seed floated. Unfortunately longer wetting time increased the difficulty of floating clover seed at a greater rate than it promoted the sinking of dodder seed. The result of several tests showed that from 30 seconds to one minute conditioning time was sufficient.

These tests show that for the separation of dodder seed from red clover seed the best factors found were to condition the seed with a 2% solution of Gardinol WA at a temperature of 80° C. for a period of 30 seconds to one minute, allowing a drying time of not more than two minutes and then separating on a bath at room temperature with a slightly reduced surface tension of about 60 dynes per cm.

The separations made by the standard Gardinol WA treatment outlined above were made with Virginia red clover seed, in which about 5% of the contained dodder seed looked abnormal. This abnormal seed was whiter and smoother than the rest of the dodder seed and was also very much more difficult to wet. There were also many shrivelled and mis-shapen seeds. The presence of this abnormal seed accounts in some tests for the failure to make a clean clover float. To determine if this condition is common to other lots of seed tests were made as shown in Table 12.

TABLE 12

Feed	Percentage floated			Dodder in float
Clover seed	Dodder seed per oz.	Clover	Dodder	
Canadian red	350	93	3	Abnormal seed
Canadian red (screenings)	5500	95	6	Nearly all abnormal
American red (screenings)	3500	70	10	Abnormal and shrivelled seeds
American red	500	68	0	
Chilean red	100	93	26	Mostly white and shrivelled seeds
Ontario alfalfa	175	91	0	

These results show that most of the dodder seeds that give trouble in the separation are abnormal. The white dodder seeds being sterile should not be counted as weed seeds. Most of the other abnormal seeds were shrivelled or mis-shapen, probably from immaturity; consequently they might be expected to be less dense than normal seed and possibly removable by gravity separation methods. As a small percentage of these abnormal seeds can germinate, however, the shrivelled seeds cannot be ignored.

Specific gravity tests with a solution of common salt, density 1.16, showed that all of the white dodder seeds and nearly all of the shrivelled seeds could be removed by a heavy solution, and suggests that if this small percentage of abnormal seeds were removed the remainder of the dodder seeds could be separated from the clover seeds.

Previously it was shown that treatment of alsike seed with common salt might be detrimental to flotation. Whether the Gardinol would change the effect of the common salt was not known, so the following runs were made using Gardinol WA as the wetting agent, and using common salt solution to remove the abnormal dodder seed. The seed used was a Canadian grown red clover containing 350 dodder seed per ounce.

(1) Common Salt Solution used on original seed. Seed washed with water and transferred wet to the flotation machine; 0.4% of total seed containing 4% of the dodder seed was removed by heavy solution. The clover seeds were brown and shrivelled. The remaining seed was slightly washed and transferred wet to the flotation machine. After flotation 96.5% of the clover seed was recovered dodder free. The sinks were about $\frac{1}{3}$ dodder and 5% of the total.

(2) Parallel tests were made drying the seed after separation by common salt and washing, and before flotation. The separation was not quite as good and 92% of the seed floated instead of 95%. Apparently the drying allowed the salt to permanently affect the seed coat.

(3) Another test in which the specific gravity separation was made on the float instead of the original seed gave a 95% float with a few dodder, 2 per oz., but a smaller quantity of seed was separated by the salt solution. The difference between this test and the first is probably due to a change in specific gravity during flotation. The salt solution does not prevent flotation in this case provided the treatment is done quickly and the salt removed by washing before it has time to soak into the seed.

While with the few lots of seed tested the use of Gardinol WA as a conditioning agent in conjunction with other controlled factors has given good results, it may be that even better reagents will be found when the present method of testing which is necessarily largely "hit and miss" gives way to other methods based on an increased knowledge of the chemistry and structure of seed coats.

Other Weed Seeds Recovered

Mixed in some of the clover seed experimented with were other weed seeds besides those particularly dealt with previously. Seed counts show that some of these weed seeds were partially removed at the same time as bladder campion, nightflowering catchfly, white cockle, and dodder seeds. Results are shown in Table 13.

There is evidently a difference in the seed surfaces suggesting the possibility that if the proper conditioning reagent or other conditions were found, the field of wet separation by surface tension might be considerably extended to cover separations which present commercial operations do not make as completely as is desired.

TABLE 13

Lambsquarters seed from alsike seed	
Lambsquarters in 1 oz. of original seed	348
Lambsquarters in 1 oz. after treatment	179
Black medic seed from sweet clover seed	
Black medic in 1 oz. of original seed	28
Black medic in 1 oz. after treatment	18
Green foxtail seeds from sweet clover seeds	
Green foxtail per oz. in original seed	230
Green foxtail per oz. after treatment	24

SUMMARY

The tests have been confined to separating weed seeds particularly from the clover seeds.

1. The experiments show that there are differences previously unknown in the wettability of the coats of seeds.

2. The differences in some cases is sufficient to allow a good separation using a water bath alone.

3. It is possible to increase the wettability of some seeds more than of others by the use of the proper reagent, and to decrease the wettability of others.

4. The difference in wettability may be increased to such an extent as to make separation difficult by other means comparatively easy by flotation methods.

5. Successful separation has been made from clover of bladder campion, nightflowering catchfly, white cockle, and dodder, and the principle may be capable of extension to other weed seeds.

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SUPPLEMENTARY FATS IN THE FATTENING RATION[1]

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In the fattening of poultry the object is to cause a deposition of fat under the skin, perimuscularly in the fascia, on the surface and between the muscles, and also internally in the body cavity. Only slight changes in the amount and character of the muscle fat are effected by the ration fed or the period of fattening. The ration fed, however, shows a marked effect on the character of the depot fats of the skin, the abdomen, and the fascia. Cruickshank (1) has reported the effect of the cereals, corn, oats, and barley, in the fattening ration on the composition of the body fat of immature and mature cockerel stock, showing harder fats being produced with the same cereal in the immature stock as compared with the mature stock. Cruickshank (2) has demonstrated the effect of supplementary fats, such as vegetable oils, on the character of the depot fats. The body fats changed in character according to the character of the oil fed in relatively short periods of time. Maw *et al* (3) have shown that the amount of fat produced by individuals varies widely under similar nutritive conditions, and that the amount of fat produced may have an effect upon the change of the colour of the fat as influenced by the feed fed. Reed, Yamaguchi, Anderson, and Mendel (4), in studying the factors influencing the distribution and character of adipose tissue in the rat, found that approximately twice as much total fat (fatty acids) was deposited by rats fed a diet rich in fat (Crisco) as by animals that ate an equicaloric amount of a diet rich in cornstarch. Similar relatively increased fat storage was found with trials using mutton tallow and soy bean oil rations. Maw *et al.* (5), studying the effect of protein levels in the ration with stock of different ages during growth, show that there is no significant effect upon the chemical composition of the thigh muscle. There was, however, a wide difference in the fat content of the muscle as between the check stock and the fattened stock.

The present problem deals with the study of the effect of yellow corn and crude corn oil as sources of supplementary fat in a mixed cereal ration upon gains with varying lengths of feeding period, and the total percentage fat found in the drawn carcass.

MATERIALS AND METHODS

Stock

Range-grown Rhode Island Red cockerels approximately mature in body development were used. Six groups of 21 individuals were fed on each ration.

[1] Contribution from the Faculty of Agriculture of McGill University, Macdonald College, P.Q., Canada, with assistance from the Dominion Department of Agriculture, Ottawa, and the National Research Council, Ottawa, Canada. Macdonald College Journal Series No. 89. Presented before the Annual Meeting, Poultry Science Association, Madison, Wisconsin, August 13, 1937.

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Treatment

All birds were individually cooped and fed, live weights and feed consumed at 7-day intervals being recorded for a total feeding period of 21 days.

Six different rations were used. The basal ration was made up of 31 pounds of each cereal: ground whole wheat, oats, and barley, 6 pounds animal protein (made up on the basis of 7½ pounds beefmeal and 5 pounds powdered buttermilk) and 1 pound salt. The basal ration was number I. Ration number II had equal parts by weight of the four cereals: ground whole yellow corn, wheat, oats, and barley, to which were added the same protein supplement and salt. Rations III, IV, V and VI, were composed of the basal ration to which were added 2.5%, 5.0%, 7.5%, and 10.0% of crude corn oil respectively as a fat supplement. The range of total per cent fat in the six rations was from 2.6% in ration number II to 12.8% in ration number VI.

PROPORTION OF BASAL MIXTURE TO SUPPLEMENT

Rations	I	II	III	IV	V	VI
Basal mixture—lbs.	100	76.75	97.5	95.0	92.5	90.0
Corn supplement—lbs.		23.25				
Corn oil supplement—lbs.			2.5	5.0	7.5	10.0
	100	100.00	100.0	100.0	100.0	100.0

PERCENTAGE COMPOSITION OF THE RATIONS

Ration number	Moisture	Ash	Fat	Fibre	Protein	N-free extract
I	11.1	4.03	2.8	5.7	14.0	62.37
II	11.3	3.68	2.6	4.4	13.4	64.62
III	11.6	4.10	5.5	5.7	13.6	59.50
IV	11.2	3.90	7.0	5.5	13.7	58.70
V	10.8	3.46	10.4	5.1	12.8	57.44
VI	10.6	3.66	12.8	5.1	12.4	55.44

Feeding

Three full-feeds daily were given throughout the entire feeding period. The consistency of feed given was such as to pour freely from a pail, but with no free water when placed in the troughs. All feed weighed back at 7, 14, and 21 days was calculated on a dry feed basis.

Preparing Carcasses for Chemical Analysis

Following dressing, the carcasses were chilled for 24 hours, after which they were drawn in the regular fashion, recording weights of all parts. The abdominal fat was considered a part of the drawn carcass. The drawn carcass was then boned, separating the skin, white meat, dark meat, and abdominal fat as individual samples for fat analysis. The perimuscular fat of the breast and leg muscles was considered a part of these two respective areas.

Chemical Analysis

The method of analysis used was that described by Holcomb and Maw (6), with the modification of allowing the digestate to evaporate to a volume somewhat less than the volume of the bulb of the mojonnier extraction tube.

THE EFFECT OF LENGTH OF FEEDING PERIOD AND CORN AND FAT SUPPLEMENTS ON FATTENING GAINS

The experimental data obtained in these trials are given in Table. 1. The mean values for initial weight are given, as well as the gains and feed consumption on a 7-day feeding period basis with their totals and the gain-feed ratio. It is to be noted that the mean initial weights for the six groups were extremely uniform. The coefficient of variability for initial weight was 8.6%. The gain-feed ratios for the total 21-day period show

TABLE 1.—MEAN VALUES OF INITIAL WEIGHT, GAIN, AND FEED CONSUMPTION

Ration	Feeding periods			
	7 days	8–14 days	15–21 days	Total
I. Wheat-oats-barley				
Initial weight	2981.90			
Gain	152.61	104.28	153.57	410.47
Feed consumed	976.00	953.52	1010.76	2940.28
Gain per 100 grams feed				13.96
II. Corn-wheat-oats-barley				
Initial weight	2937.61			
Gain	144.52	151.19	101.42	397.14
Feed consumed	943.19	989.33	987.90	2920.42
Gain per 100 grams feed				13.59
III. Wheat-oats-barley + 2½% corn oil				
Initial weight	2969.04			
Gain	209.28	123.33	132.14	464.76
Feed consumed	1037.71	985.57	1013.95	3037.23
Gain per 100 grams feed				15.30
IV. Wheat-oats-barley + 5% corn oil				
Initial weight	2938.57			
Gain	160.47	139.28	116.66	416.42
Feed consumed	971.04	975.42	947.33	2893.80
Gain per 100 grams feed				14.39
V. Wheat-oats-barley + 7½% corn oil				
Initial weight	2908.33			
Gain	206.19	113.33	126.66	446.19
Feed consumed	996.66	961.90	954.00	2912.57
Gain per 100 grams feed				15.31
VI. Wheat-oats-barley + 10% corn oil				
Initial weight	2969.76			
Gain	160.23	141.42	129.04	430.71
Feed consumed	913.90	985.61	935.47	2835.00
Gain per 100 grams feed				15.19

slight differences, the rations supplemented by oil having slightly higher ratios of feed consumed to the gains made.

For the purpose of analysing the gains on the basis of variance, the 21 birds on each ration were randomized into three groups, fed for 7, 14, and 21 days. In Table 2, the analysis of gain is given on a basis of variance due to periods and rations within periods. This analysis shows the significant effect of the longer feeding periods on gain. There was no effect of the different rations on the gains made, the mean square between rations within periods being somewhat smaller than the mean square within rations. The coefficients of variability of gain for the 7-, 14-, and 21-day periods were 83.8%, 43.8%, and 40.3% respectively.

TABLE 2.—ANALYSIS OF GAINS

Source	D/F	S.S.	M.S.	$\frac{1}{2}\log_e$	S.D.
Between periods	2	1,596,988.49	798,494.25	6.7952	
Between rations within periods	15	314,132.74	20,942.18	4,9748	
Within rations	108	2,540,846.43	23,526.35	5.0328	153.58
Total	125	4,451,967.66	z for periods = 1.7624		

It would appear that the gains made on a short-term feeding period are very greatly influenced by the initial reaction of the individual birds to the environmental and feeding differences of the fattening method. With the longer feeding periods, the birds made much more uniform gains.

In order to eliminate the effect of initial weight and feed consumption on gain, the observed gains have been corrected by regression. The analysis of the adjusted gains is given in Table 3. This analysis shows that even with the removal of the effect of these two variables no differences exist between the gains made by the birds on the different rations.

TABLE 3.—ANALYSIS OF ADJUSTED GAINS

Source	D/F	S.S..	M.S.	$\frac{1}{2}\log_e$	S.D.
Between periods	2	11,394,802.52	5,697,401.26	7.7777	
Between rations within periods	15	196,932.02	13,128.80	4.7409	
Within rations	106	1,101,338.51	10,389.99	4.6188	101.93
Total	123	12,693,073.05			

. Corrected for regression of gain on initial weight $b_1 = -0.0278431$ and gain on feed consumption $b_2 = 0.5056757$.

RESULTS OF CHEMICAL ANALYSIS

Table 4 gives the percentage of fat found in the analysis of the carcasses of three birds (drawn at random) from the groups fed on the different rations. It can be seen that the percentage of fat in the different parts is quite uniform for all the rations except ration number II, which contained corn, wheat, oats, and barley. In this case the percentages are slightly

higher. The raw data show that individually the three birds of this group, used in the analysis, were above the level of the birds in the other rations. The gains made by these birds were but slightly greater, and the feed consumed slightly less, than the average for the twenty-one birds.

The variance analysis of the percentage of fat shows significant differences between the ration groups. As shown in Table 5, the z value obtained is 1.0691. This is greater than the highly significant point of z for $n_1 = 5$ and $n_2 = 10$ which is 0.6009. In Table 6 the observed mean

TABLE 4.—CHEMICAL ANALYSIS OF MEAT, SKIN AND ABDOMINAL FAT. PERCENTAGE FAT IN SAMPLE

(Average of three individuals for each ration)

Ration	Skin	Abdominal fat	White meat	Dark meat	Total meat
I	32.53	77.03	3.79	8.53	6.55
II	47.06	88.26	4.82	9.13	7.18
III	42.50	80.56	3.80	6.26	5.12
IV	35.60	77.80	3.53	6.21	5.03
V	39.20	81.66	3.97	7.40	5.86
VI	38.50	82.36	4.03	7.93	6.25

TABLE 5.—ANALYSIS OF VARIANCE. PERCENTAGE FAT IN DRAWN CARCASS

Source	D/F	S.S.*	M.S.	$\frac{1}{2}\log_e$	S.D.
Between treatments	5	147.803	29.561	1.6932	
Within treatments	10	34.840	3.484	.6241	1.866
Total	15	182.643		$z = 1.0691$	

* Sum of squares corrected for regression of percentage fat on initial weight $b_1 = -0.00316954$ and percentage fat on feed consumption $b_2 = 0.00243345$.

percentages of fat in the total carcass are given, together with the values obtained when corrected by regression. From the standard deviation of 1.866 the necessary difference for high significance between any pair of means is 4.6536. It can be seen in Table 6 that the birds fed ration II,

TABLE 6.—MEAN VALUES OF PERCENTAGE FAT IN CARCASS

Ration	No. of birds	Observed	Corrected by regression
I Wheat-oats-barley	3	10.25	10.39
II Corn-wheat-oats-barley	3	16.77	17.22
III Wheat-oats-barley + 2½% corn oil	3	11.44	10.66
IV Wheat-oats-barley + 5% corn oil	3	10.09	9.47
V Wheat-oats-barley + 7½% corn oil	3	11.26	11.17
VI Wheat-oats-barley + 10% corn oil	3	11.66	11.97

containing the cereal corn, had a significantly greater amount of fat than any of the other groups. The differences between the birds fed the other rations are slight and of no significance.

SUMMARY

No significant differences in gain were found between birds fed on a basal fattening ration of wheat, oats, and barley, and five lots fed on the same basal ration supplemented by the cereal corn and varying amounts of corn oil.

Greater gains were made with 14-day and 21-day feeding periods as compared to a 7-day feeding period. The gains made during the shorter period had a very high coefficient of variability.

From the chemical analysis of representative samples drawn from the various groups it was found that corn, when added to the fattening ration, increased the percentage fat in the body tissues, and that corn oil added in varying amounts to the same basal ration did not increase the percentage of fat.

ACKNOWLEDGMENT

The corn oil used in these trials was supplied through the courtesy of Canada Packers Limited, Montreal.

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BOOK REVIEW

Armstrong, S. F. British Grasses and Their Employment in Agriculture. (Third Edition). Published by the Cambridge University Press. Macmillan Company of Canada, Limited. $5.00.

This revised and enlarged edition includes much of interest to the practical agriculturist as well as to the teacher of scientific agriculture and to those engaged in agricultural research. The material is divided into two main parts—first a botanical section and second an agricultural section. A unique feature is the inclusion of the index with the glossary.

The Botanical Section opens with a chapter on morphology, in which the grass plant is considered from the root to the seed. The second chapter deals with the physiology of the grasses. Next follows a grouping of the principal species according to habitat and this is followed by three keys based on vegetative, floral and seed characters. The botanical section is completed with descriptions of about one hundred species.

The Agricultural Section opens with a chapter on "Characters which determine the use and relative value of grass in agriculture"; next follows a discussion of commercial, indigenous and new strains. Sixty pages are devoted to a discussion of the economic uses of about thirty of the more important species. Such points as suitability for grazing or for hay, type of turf produced, palatability, whether a "top" or "bottom" type, and season of high production are discussed in some detail.

The remaining chapters of the agricultural section deal with grass production including establishment and management of new stands of grasses and improvement of poor stands, the advantages of mixtures as compared to pure cultures, soil preparation, methods of sowing, use of nurse crops, use of fertilizers on different species, effects of over- and under-grazing, haymaking, ensilage and artificial drying of grasses.

Throughout this book the author has been concise yet descriptions and discussions are complete. The clear style of writing makes the text easy to understand and a pleasure to read.

—T. Stevenson.